THE EFFECT OF CERTAIN LEVELS OF IRON, COPPER AND ETHYLENE DIAMINE TETRA-ACETATE IN A PIG RATION ON LIVER AND SPLEEN IRON CONCENTRATIONS

by

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TABLE OF CONTENTS

INTRODUCTION

Ancient Egyptians and later the Greeks and Romans are said to have had experience with iron treatment, having had some knowledge of its therapeutic properties. In the last century, detailed balance studies and experiments using isotope labeled compounds, have somewhat clarified the requirements for iron in mammalian systems. As one of the essential trace elements, iron is involved in oxygenation processes as a component of hemoglobin and myoglobin, and is a vital component of various oxidative enzymes involved in cell metabolism.

Various treatments involving iron, copper and certain chelating agents have been studied in animal and plant iron-deficiency states. Anemia is a world-wide nutritional problem and has been reported to be second only to protein malnutrition in numbers of people affected. Iron deficiency has become a paramount concern in the swine industry since housing of animals indoors on concrete became the common practice. Iron deficiencies in various plants have been studied in detail. Copper, which is concerned with utilization of iron, and chelating agents which form complexes with various metals have been shown in both animal and plant studies to affect iron absorption and utilization.

The purpose of the work herein reported was to study the effect of various levels of iron, copper and ethylene diamine tetra-acetate (EDTA) in the ration on concentrations of iron in the liver and spleen of pigs. Since the liver and spleen are the chief storage depots, the iron levels were determined in these

organs and the results obtained were used to compare effects of the various rations.

REVIEW OF LITERATURE

Role of Iron in Mammalian Biological Systems

Importance and Distribution of Iron. Because of the world-wide prevalence of anemia in humans (1) and the fact that it is the most common nutritional deficiency disease in children in this country (2), studies of iron requirements, functions, deficiencies, toxicity and treatments are important. Pigs are the only farm animal seriously affected by iron deficiency (3, 4, 5, 6), and this condition has become a major economic concern in this industry.

Iron requirement seems to be a function of growth and of turnover of red blood corpuscles (RBC); thus fast-growing mammals need more iron than do mature ones (7). The requirement for young mammals varies with species and age. The adult human requirement of 1-2 mg can be provided by an average 10% absorption of a 15-mg daily intake (8). The distribution of the 4-5 g of iron in the normal adult human body is: hemoglobin (Hb) 70-73%, ferritin and hemosiderin 16-17%, myoglobin 3%, transferrin 0.07%, and the remainder in respiratory enzymes and in plasma (9, 10).

Hemoglobin. The major metal-containing chromoprotein in vertebrates is Hb. It is a simple globin with a ferrous iron porphyrin, heme, (3) which contributes the color to RBC (11). Synthesis of Hb takes place in bone marrow with porphyrin

biosynthesis producing the heme, and protein biosynthesis providing the globin (12). Iron may come from storage depots or from destroyed RBC. When the RBC are broken down by macrophages in the liver, red bone marrow and spleen, Hb is freed and split into the globin and heme portions (11). The heme portion is broken down further to release iron and bilirubin, the yellow coloring matter in bile. This released iron is then available for new RBC formation or it may unite with protein to form storage ferritin. Copper is essential for normal Hb synthesis although it does not become a constituent (3, 13, 14, 15, 16, 17, 18, 19). The action of copper may be as a catalyst for the synthesis (19) or as a mobilizer of iron (16).

Because of the large proportion of total body iron present in Hb and because it is probably one of the first iron-containing tissues to be depleted as a result of deficiency, the Hb level has been used as an indication of adequate iron supply (20). Drabkin (10) has used 12 g and 11 g Hb/100 ml blood or below as indicative of anemic conditions in adult males and females, respectively. Normal levels for these individuals are 16.0 g and 14.5 g Hb/100 ml blood, these differences between the sexes usually being consistent after puberty.

Hemoglobin functions as an oxygen carrier. When blood, or Hb, is mixed with air, the oxygen unites with the iron to form oxyhemoglobin or oxygenated Hb (11). The transport and delivery of oxygen to tissues is dependent upon a loose, reversible combination and should not be confused with oxidation in which the valence state of iron is changed and methemoglobin is formed.

Ferritin and Hemosiderin. The 2 non-heme protein complexes containing storage iron are ferritin and hemosiderin (2, 21, 22, 23). The relative distribution of iron between the 2 forms appears to be dependent upon total iron concentration (22), with the ferritin as the active or labile storage form and the hemosiderin the less active form probably present only when excesses of iron occur (10).

Ferritin, a stable, soluble, non-toxic compound is composed of iron and a colorless, homogeneous protein called apoferritin (24). Ferritin is 23% iron by dry weight (11, 18, 23, 24). This iron, which is probably on the surface of the protein molecule (24), may be absorbed from food in the gastrointestinal (GI) tract (18) or from breakdown products of Hb. Hahn et al. (25) demonstrated in dogs that ferric ammonium citrate administered by vein was readily converted to ferritin in the liver and that destruction of RBC in these dogs by acetylphenylhydrazine allowed at least part of the Hb iron to be converted into ferritin in the liver and spleen.

A hypothesis advanced by Mazur and workers (26) explained the role of ferritin in iron transport and heme synthesis based on oxidation and reduction mechanisms. Their data show a small proportion of total iron is reduced to the ferrous state producing sulfhydryl ferritin. This portion, at or near the surface, is readily available to iron-binding plasma protein and is easily dissociated in the presence of a suitable iron-binding agent. However, Mazur et al. (26) reported that the bulk of the iron in ferritin appeared to be unavailable for this reaction because it

is in the ferric state. The possible role of ferritin in iron absorption regulation will be discussed.

Finch (21) has described the 2 important localities of iron storage. The first, the reticulo-endothelial, includes fixed phagocytic tissue of the reticulo-endothelial system and the wandering macrophages. The second is the parenchymal, made up of polygonal cells of the liver, and glandular tissue of the pancreas, adrenal and other secretory cells in the body. The liver is commonly studied because of the presence of extensive reticulo-endothelial network. The spleen represents reticulo-endothelial tissue with special implications related to blood cell breakdown. These 2 organs are the chief iron depots in mammalian systems (3). The kidney also has the ability to take up Hb iron (21), and it has been shown to aid in conservation of iron when Hb injections have produced levels that the liver alone cannot absorb (24). The heart, skeletal muscle, pancreas and brain contain some storage iron but are not considered storage depots (3).

Iron storage has been shown to vary with age and overall condition of the body. Storage, mainly as ferritin, is high at birth and then falls to a low level during infancy (22). It remains at this low level until growth has ceased, usually about age 20, and then increases to a higher level which may be maintained throughout normal adult life. Sometimes it drops after 50-60 years of age. Low stores follow excessive losses of blood which require utilization of storage iron for Hb synthesis.

Unusually high storage may result when the body's ability to excrete does not keep up with absorption (21, 27), or in cases of aplastic anemia, multiple myelomae, leukemia, blood transfusion or during a redistribution of iron from the blood to stores (22).

Myoglobin. Myoglobin is the second major chromoprotein. It is closely related to Hb, but contains only 1 iron group as compared to 4 in Hb (10). This intracellular pigment is responsible for storage of oxygen in red muscles (3, 10, 11).

Transferrin or Siderophilin. Transferrin, also called siderophilin, is an intensely colored B_1-globulin and iron complex with each protein molecule binding 2 Fe^{III} ions (12, 28). Although Cu^{II} and Zn^{II} can also be bound to the protein in a ratio of 2 metal ions to 1 protein molecule, their affinity is much less than Fe^{III} (28). Iron from foodstuffs or from destroyed erythrocytes may be picked up by the protein and carried in portal blood to storage depots or to bone marrow (12). Once bound, the transferrin is not split at physiological pH ranges, but when it has reached the utilization site, iron is released and the carrier is free to pick up more iron.

Cytochrome System. Cytochromes, present in cells of nearly all aerobic plant and animal tissues, are the most widely distributed of all heme proteins (29). The content in tissues varies, but it parallels respiratory activity with the heart having the highest concentration. Although it has been understood for some time that reduction of oxygen in cellular respiration is accompanied by formation of high-energy phosphate compounds, there still is much to be learned about the mechanisms involved

(30). The respiratory chain as illustrated by Chance in 1958 is as follows:

$$AH_2 \longrightarrow \text{cyt.b} \longrightarrow \text{cyt.}c_1 \xrightarrow[B]{} \text{cyt.c} \xrightarrow[A]{} \text{cyt.}(a+a_3) \longrightarrow O_2$$

Disruption at A yields cytochrome oxidase. Splitting at B and A produces cytochrome c.

Cytochrome b differs from the others in that it is autoxidizable (29, 31). Cytochromes a, c, and c_1 can be oxidized by molecular oxygen only in the presence of cytochrome oxidase. Because oxidation of cytochrome b is not dependent on the presence of oxidase, it is not totally inhibited by cyanide as are the others, but narcotics will cause inhibition. Cytochrome oxidase, an almost universally distributed enzyme, is identical with indophenol oxidase and Warburg's respiratory enzyme (31). It appears to be a hemochromogen-like compound and may even be cytochrome a_3. As the terminal member of the chain, the cytochrome oxidase is understood by White et al. (32) to be the only member capable of reducing oxygen. Cytochrome c is the limiting factor in autoxidation of cytochrome oxidase (10, 32). The exact oxidation and reduction mechanism is not entirely understood but is presumed to involve a gain of 1 electron since the only difference between oxidized and reduced forms is observed in the valence state of the iron present (31). That is the passing from the ferric to the ferrous state upon reduction and is analogous to the change involved when methemoglobin is reduced to hemoglobin.

Catalase and Peroxidase. Catalase and peroxidase are enzymes found in animal and plant tissues, with the latter much more common in plant than in animal cells (29, 31, 32). Catalase, a ferriprotoporphyrin present in all aerobic organisms, catalyzes the reaction of hydrogen peroxide breakdown to water and molecular oxygen. It has been crystallized from beef liver and found to contain 0.1% iron (29). This enzyme is specific and does not split peroxides except in concentrated solutions (31). One unit of beef liver catalase can split 44,000 molecules of hydrogen peroxide per second (32).

Peroxidase content in plants is often high, but concentrations in animals are relatively low (31). This enzyme catalyzes the decomposition of hydrogen peroxide also. Peroxidase action differs from that of catalase in that a substrate which becomes oxidized as a result of the split is involved (29). Baldwin (31) reported that most heme and hematin derivatives, including free hematin, possess a weak peroxidase activity.

Some Other Iron-requiring Compounds. Certain flavin enzymes contain iron whereas other enzymes seem to require its presence in a reaction (32). Succinic acid and nicotinamide adenine dinucleotide (NAD) dehydrogenases are non-autoxidizable flavins containing iron. Dihydroorotic acid dehydrogenase, aldehyde (liver) oxidase and xanthine (milk) oxidase are autoxidizable iron-containing flavin enzymes. Xanthine oxidase catalyzes the oxidation of hypoxanthine $\longrightarrow$ xanthine $\longrightarrow$ uric acid, as well as many oxidations of aldehydes to their corresponding acids. Aconitase is believed to require the presence of iron for the

reversible interconversion of citric, cis-aconitic and isocitric acids.

Iron Absorption. Iron absorption has been shown to take place at any level of the GI tract, but mainly in the duodenum, decreasing progressively descending the tract (33). The pattern for absorption is a discontinuous one with a rapid phase during the first hour and a slower one up to 12-24 hours following ingestion (34). Within 2 hours, 60-80% of the total iron absorption has been accomplished.

A number of factors including growth, state of body stores, and rate of erythropoiesis affect iron absorption (33, 35). Iron absorption efficiency in normal adults is about 10% (27, 36). Schulz and Smith (2) reported most children in their study absorbed at least 5 times more iron than did the adults observed. Pregnancy, deficiency anemia, and some stages of hemochromatosis may cause increased absorption (33), whereas an overload condition in tissues, diminished erythropoiesis, and certain malignant, inflammatory or infectious diseases may cause decreased absorption. Prolonged diarrhea is detrimental to iron absorption because the chyme does not remain in the intestine long enough for normal absorption (18).

The form of the ingested iron may affect its absorption. Moore and workers (37) have demonstrated differences in absorption rates for ferrous and ferric iron in humans and dogs. The amount of ferrous iron absorbed ranged from 1.5-15 times the amount of ferric iron absorbed by normal human subjects. They suggested the possibility of ferric ions forming insoluble compounds in the

GI tract which make them unavailable for absorption. Dogs were reported to absorb both forms equally well. Pigs probably absorb the ferrous form more readily than the ferric.

Types of food containing iron and those foods eaten with iron-containing ones also may have an effect on absorption. The iron from heme compounds as in meats may not be available as the iron porphyrin is not split to release iron for absorption (3, 18). Ascorbic acid, as in citrus foods, or other reducing agents may aid absorption by converting ferric iron to the ferrous state.

Iron metabolism, a one-way process, seems to involve 3 steps (21, 38). These are: 1) iron uptake by mucosal cells of the intestine; 2) iron transfer from mucosal cells to the plasma iron-binding protein; and 3) iron delivery to various tissues in the body. Since negligible amounts are excreted, a regulating mechanism must exist. The most widely accepted theory is that of Granick (39) which is based partially on work by Hahn (25).

In 1943, Hahn et al. (25) studied iron absorption by the GI tract in growing dogs using radioactive iron. It was found that an ordinary iron dose given 1-6 hours before radioactive iron produced what they termed a "mucosal block." Uptake of radioactive iron was less than expected. Iron given some days before the radioactive iron did not appear to inhibit iron absorption. The term "physiological saturation" was used to explain acceptance or refusal of ingested iron by GI mucosal epithelium. They found that desaturation took place over a matter of days rather than hours, whereas saturation was accomplished within 1-2 hours. It was concluded that the complex protein metabolism of the cell

may be involved in this saturation change.

Granick (39) in 1946 enlarged upon Hahn's theory by studying the function of ferritin as the regulatory mechanism in iron absorption control. As has been previously suggested, iron is absorbed in the ferrous state and excess is converted to the ferric state and temporarily stored as ferritin in the mucosa. The hypothesis suggested by Granick stated that an equilibrium relationship exists between ferritin iron and mucosal ferrous iron. When the cell is "physiologically saturated" with ferrous iron, absorption of iron from the tract stops. Parallel to the saturation, then, are the "mucosal block" or resistance to further iron absorption and the rapid increase in ferritin content. Only as ferritin iron has decreased causing the mucosal cells to become desaturated with respect to ferrous iron would iron be absorbed again from the gut.

In later work by Granick (24), 1951, 2 mechanisms involved in iron absorption regulation were discussed. The "mucosal block" with its direct or indirect connection with ferritin content was the first mechanism. The second consideration was for the "redox" level of the cell. Since ferrous ions diffuse into the blood stream, conversion of the ferric hydroxide micelles of ferritin to ferrous ions is dependent on lower oxygen tension in the blood. In anemic conditions where oxygen tension is low, absorbed ferrous iron remains reduced and passes directly through the cell into the blood stream.

Beutler et al. (40) concluded from their work that the same mechanisms are active when small or massive doses of iron are

given. They suggested that acute iron poisoning may not be due to leakage of toxic inorganic iron across the membrane because of some destruction of the intestinal mucosa, but rather due to an extension of normal absorptive processes into the lethal range.

Iron Deficiencies. Iron deficiency anemia occurs when total body iron is insufficient to provide a normal mass of Hb per unit volume of blood (18). This results in reduced oxygen carrying power in the circulation. Causes of anemia vary. Excessive blood losses as in severe hemorrhage or slow continuous bleeding of an ulcer, hookworm infestation, destruction of corpuscles as in malaria, or dimunition in production of RBC may result in anemia (11). Anemia attributed to lead poisoning is the result of decreased life span and abnormal production of RBC (12). There is increased erythropoieses, much of which is ineffective, and elevated serum iron probably resulting from increased hemolysis and defective utilization of iron. Pernicious anemia is not due to lack of Hb but results when erythrocytes in bone marrow do not mature properly (11). This condition involves an extrinsic factor (vitamin B_{12}) and an intrinsic factor present in normal gastric juice.

Iron deficiency induced in rats produced animals that were lethargic and fell asleep easily, although they were playful when handled (41). The coats were shabbier than normal, hair grew unevenly, eyes had a transparent pallor and teeth were pearly white in contrast to the golden yellow color of those receiving iron supplement. Feces were poorly formed.

In Aberdeen, the McGowan group (42, 43) recognized iron deficiency as the cause of ailment and death of many pigs when sows were farrowed indoors in concrete floored pens. Although pigs looked well until 3-4 weeks of age, they soon became dull and listless. Skin became intensely white and the animals developed a hairy appearance. Edema of the skin gave them a thick-set look. Breathing was in "thumps" with spasmodic jerking of the diaphragm and sudden respiratory convulsions usually resulting in death. Post mortem examination revealed fatty changes in the center of liver lobes and advanced fatty degeneration of the heart and of the epithelium of secreting tubules of the kidneys. The heart was dilated, filling most of the chest cavity and causing the lungs to be crushed back. The liver was pale and had minute paler areas all over the surface; the pancreas was extremely white; the kidneys were pale; and the spleen was usually slightly enlarged. The few animals that lived were emaciated, ceased to grow, became hairy and the skin became dry, dirty and acquired a cinnamon tint. When iron deficiency was recognized as the cause of the ailment, ferric oxide was added to the ration; animals became more lively; and appetites and Hb levels increased. The iron treatment could not remove all of the disease symptoms such as organic changes in the liver and lungs, but it did have a marked effect in removing fatty changes.

Hart et al. (44) in 1925 studied various rations to determine effectiveness of iron in the nutrition of rabbits. They found that rabbits developed anemia when limited to cows' whole milk-sodium citrate ration, or this ration with added ferric

oxide. When the inorganic iron was given with alcoholic extracts of yellow corn meal or cabbage or in the presence of fresh cabbage, cure or prevention of anemia was observed.

As late as 1930, data still showed about 35% of the pigs farrowed were lost during nursing (45). Many factors were involved but anemia was the most prevalent cause of death. Hart et al. (45) found that liberal iron and copper additions to sows' rations were not sufficient to prevent anemia development in young suckling pigs. They suggested direct feeding of soluble iron salts. This was done using cow's milk with or without the addition of copper. As soon as iron was supplied, Hb synthesis proceeded since iron reserves were probably depleted first. Gehle et al. (5) concluded in 1961 that for prevention of iron-deficiency anemia, pigs raised on concrete without access to soil or creep feed need iron supplementation of some type.

Iron Supplementation. Since iron deficiency was recognized as the cause of disease prevalent in pigs, several treatments for prevention and cure of the ailment have been studied. McGowan et al. (42, 43) added ferric oxide to the ration and noted a marked effect on the condition of the animals. Hart et al. (45) fed pure ferric chloride in cow's milk and reported recovery to be almost as rapid as when both iron and copper were added. Ullrey et al. (46) suggested 125 ppm oral iron with 25 ppm being supplied by the ration and the remaining 100 ppm added as ferrous sulfate as adequate for the baby pig. Matrone et al. (47) reported the minimum iron requirement of baby pigs up to 60 days of age to be approximately 60 ppm of the dry matter

intake on a fortified cow's milk ration. The difference between recommendations may be attributed to difference in composition of the dry ration.

Treatment of sows may or may not affect iron intake by baby pigs. Chaney and Barnhart (6) were unable to increase placental transfer with graded levels of ferrous fumarate up to 1382 mg/kg, but they did note iron in sows' milk was maintained at levels to prevent anemia. Maner et al. (48) sprayed a saturated ferrous sulfate solution on sows' udders daily. This was effective only in preventing extremely low Hb levels. Rydberg et al. (49) gave intramuscular injections of iron-dextran to dams 4 weeks prior to farrowing but noted little difference between pigs from untreated and treated dams. This may have been attributed to giving injections too early in the gestation period.

Because absorption efficiency for iron is low, there has been a search for a non-toxic compound that would release iron satisfactorily for use by the animal system (50). Intramuscular injection of an iron-dextran solution was suggested as a practical means of preventing and treating anemia in baby pigs. Most workers agreed that 100 mg elemental iron in iron-dextran injections were successful in improving anemic conditions although maximum Hb synthesis did not result at this level (48, 50). Significant Hb increases and weight gains were not maintained as well over a period with 100 mg as when larger doses were used (48, 51, 52, 53). The 100-mg treatment with iron-dextran had a decided advantage over oral tablets (50), oral paste and no treatment (53). Doses up to 1000 mg elemental iron given in

2 injections over a 1-week period have been tried with no toxicity reported (48). Injections of peptonized iron solution (250 mg iron) resulted in 37% mortality when administered to pigs at birth (52). The injections did not maintain Hb levels in surviving pigs. When a second injection was given at 21 days, there were no deaths but Hb levels were still significantly less than those in pigs receiving iron-dextran injections.

Various oral methods have been studied but usually without the success reported for intramuscular injections of iron-dextran. Maner (48) administered a pill containing 292 mg ferrous iron, 21.6 mg copper sulfate and 2.85 mg cobalt sulfate at 3 and 10 days. Mild scouring, coughing up of undissolved pills and low Hb levels were reported. Wahlstrom (52) using a similar pill at birth, 10 and 21 days noted a decrease in Hb level from 11.03 g/100 ml blood at birth to 8.39 g at 10 days. The Hb level was then maintained around 8 g/100 ml. An oral paste containing 40 mg iron as ferrous sulfate and 2.5 mg copper as copper sulfate given at birth and twice weekly thereafter for 8 weeks gave a favorable Hb level response, and at 5 weeks these pigs slightly surpassed the pigs which had received 100 g iron as iron-dextran at birth (53). The major limitation of oral treatment as seen by Zimmerman et al. (53) is the necessity for repeated administration throughout the suckling period.

There are other benefits of supplementation of a ration with minerals besides prevention and cure of anemia. Increased growth rate was reported in addition to increased Hb synthesis in pigs (5, 54, 55). Copper was shown to significantly increase growth

rate, rate of food consumption and dressing percentage and to decrease carcass length (55, 56). Hart et al. (19) noted improved physical well being, increased appetite, smoother coats and increased vigor and activity when copper sulfate was added to the rat ration. Rapid Hb synthesis was reported by Mitchell and Miller (17) when copper was fed with iron to rats. Bray et al. (57) reported faster gains and higher quality veal when whole milk supplemented with copper and iron was fed to calves than when calf starter rations or rations limited in milk were fed. Darker colored meat was noted when additional iron and copper were fed.

Iron Toxicity and Overload Conditions. A problem with iron toxicity has been noted since administration of various iron salts for prevention and cure of anemia became common for humans. By the early 1950's the toxicity of frequently prescribed ferrous sulfate drugs had become a concern in the medical profession (58). Dominant symptoms of acute intoxication from ferrous sulfate were reported in 3 human cases. The symptoms were vomiting, clear watery stools which later became tarry, shock-like appearance and collapse, rapid, weak or imperceptible pulse and low blood pressure. Also, hypotonia and hyporeflexia were noted. It seems that a 3-10 g dose of ferrous sulfate is fatal to children (59). Reporting on a fatal case of ferrous sulfate poisoning, Swift et al. (60) indicated their findings were in agreement with earlier reports of 2 deaths in U. S. literature and 5 in English literature. Death occurred 24-48 hours after onset of illness. Post mortem examination revealed hemorrhages, necrosis in the

stomach and small intestine, degenerative changes in the liver, hemorrhagic bronchopneumonia and evidence of widespread bleeding. They emphasized the biphasic clinical course of initial shock followed by an intermediate period of relative recovery in response to gastric lavage, blood transfusion and general supportive therapy, and then sudden death.

Reissmann et al. (59) studied absorption of toxic doses of iron in rabbits and mongrel dogs with solutions of ferrous sulfate, chloride and gluconate. Because therapeutic doses are thought to be absorbed predominately in the duodenum, they were surprised to find toxic doses rapidly absorbed from the large bowel. There was little iron excretion in the urine. The mucosa from both the small intestine and the large bowel was reported to be histologically intact. The lethal dose was around 200 mg iron/kg body weight, and survival time varied inversely with the administered dose.

In a study of effects of absorbed iron salts from toxic doses, Reissmann and Coleman (61) reported profound metabolic acidosis, greatly increased respiratory rate with greatly diminished volume, lowered blood CO_2, excessive output of CO_2, decreased cardiac output, congestion in capillaries, and increased permeability of capillaries. Causes of acidosis were the hydrolyzing effect of ferrous ions and a possible interference with Krebs cycle enzymes resulting in increases of lactic and citric acids. Wilson et al. (62) reported interference in blood coagulation when rabbits received 400 mg or more iron/kg body weight. There was a prolongation of coagulation time which was

proportional to the administered dose. The most marked effect was on the fibrinogen. As the physiologic activity of this was decreased, the coagulation was prolonged or completely absent.

The question of toxicity may be raised when iron supplementation of pig rations is recommended to prevent or cure anemia. As indicated earlier, injections of peptonized iron solution resulted in 37% mortality when administered to pigs at birth (52). O'Donovan et al. (63) studied various rations to determine toxic levels of dietary iron, to characterize symptoms of toxicity, and to study interrelationships between toxic levels of iron and different phosphorous levels. A basal, semipurified ration containing 15 ppm iron was compared with those containing additional iron as ferrous sulfate. Pigs fed 1000 ppm iron or over had dark colored feces, the color intensity increasing with iron levels. When a corn-soybean meal ration was supplemented with iron at levels from 80-5000 ppm, animals receiving 3000 ppm or less performed well. Those receiving 4000 ppm gained significantly less ($P < 0.05$) than controls, whereas the ones on 5000 ppm had significantly depressed ($P < 0.01$) serum inorganic phosphorous and femur ash when compared to controls. Rickets occurred by the fourth week in animals fed 4000 ppm iron. Symptoms were incoordination and weakness in the hind quarter, shifting on hind feet or sitting at the feeder, obvious signs of pain when disturbed, humped back and bowing out at femurs. High levels of dietary iron were found to be more toxic when the ration contained 0.3% phosphorous than when it contained either 0.6 or 1.2% phosphorous.

Iron Overload Conditions. An iron overload or exogenous hemochromatosis may be induced by dosing with iron orally, by parenteral injections or by multiple transfusions as for hemolytic, aplastic or pernicious anemia (10, 27). Hemochromatosis may be due to excess deposition of iron, mainly as hemosiderin, as in "hemosiderosis" or "cytosiderosis." Hemochromatosis is a rare heritable disease found mainly in males (10, 24, 27). It is an inborn error of metabolism in which there is a failure of the "mucosal block" to prevent unneeded iron. This may be a result of increased effectiveness of reducing enzymes or decreased effectiveness of oxidizing enzymes.

Copper and Its Interrelationships with Iron

Copper and Its Metabolism. Copper, a trace element found in all living matter, is found most abundantly in the liver, spleen and kidneys of mammals (18). Concentrations of copper in whole bodies vary with species, but generally for all are highest in the newborn, remain high during the suckling period, and then fall steadily from weaning until the normal adult level, approximately 100-150 mg in humans, is reached (3).

Copper absorption is not as well understood as is iron absorption. Underwood (3) has indicated that the upper small intestine probably is the site of absorption in humans, whereas the upper jejunal loop rather than the middle or distal portions in dogs is the absorption site. Excretion in all species is chiefly through the intestinal tract, with 90% or more appearing in feces.

Dempsey et al. (64) demonstrated the influence of dietary intake of copper by rats on levels in serum and liver. They reported prompt decrease in serum copper with slower and less extensive liver copper depletion upon dietary restriction. High dietary levels of copper resulted in rapid increase in serum copper during the first 20 days, followed by an even greater increase in liver copper.

Copper-Iron Interrelationships. Hart et al. (19) in 1928 made their first attempt to correct anemia with 0.5 mg ferric chloride and 0.25 mg copper sulfate. Working first with 1 rat, they noted an increase in Hb level from 2.68 g/100 ml blood to 9.35 g during the first 2 weeks following treatment. This improvement continued until a normal Hb level was attained. In further studies with various levels of copper and iron, it was found that 0.1-0.5 mg copper as copper sulfate resulted in rapid recovery and even 0.01 mg copper showed marked improvement with eventual return to normal. With Hb recovery, the rats showed greatly increased appetites, smoother coats and increased vigor and activity.

By 1931, Mitchell and Miller (17) had reported studies with various levels of iron, copper and manganese supplements. They confirmed the reports of rapid synthesis of Hb with traces of copper. An optimum for rats was between 0.1 and 1.0 mg daily, with no added increase in Hb regeneration above 1.0 mg; but a slight, although distinct, retardation with copper levels below 0.1 mg if iron was as low as 0.25 or 0.1 mg daily.

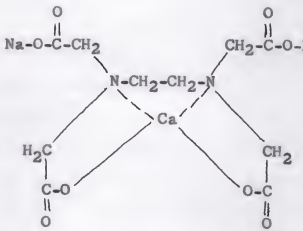
It is now generally agreed that copper is necessary for the utilization of iron although it is not itself a component of Hb. Cook and Spilles (16) suggested the involvement of copper in the mobilization of splenic iron, and Hart et al. (19) suggested a catalytic involvement in some Hb synthesis reactions. Maass et al. (15) supported this theory with a dog experiment. After rendering dogs on a raw milk diet anemic by bleeding, they fed 10, 20 or 30 mg iron and 3 µg copper to bring about immediate increases in Hb, hematocrit and red cell counts.

Significant increases in weight gain, rate of food consumption, and dressing percentage with decreased carcass length were reported by Barber et al. (56) when copper sulfate was added to control rations of pigs. Requirements for copper by turkeys were found to vary, depending on levels of iron being fed (65).

Chelating Agents and Their Influence on Iron Metabolism

Chelating Agents. Recently, chelating agents have been studied with special attention focused on their ability to mobilize certain metals. The term "chelate" comes from the Greek word "chele" for claw because polydentate ligands grasp the central ion, usually a metal ion, from at least 2 sides (66). Practically all such complexes are a 1:1 ratio regardless of metal valence. Ethylene diamine tetra-acetate (EDTA) is one of the more commonly used chelating agents which forms soluble non-ionizable complexes with certain polyvalent ions (67). These non-colloidal, organic substances (68) bind a variety of metals in definite order, depending on factors such as pH and stability

constants for the individual metal chelates (69, 70). Welcher (70) outlined the complexing and ionizations of various metals with EDTA. Complexes with divalent cations are very stable in basic to slightly acidic solutions, while those with trivalent metals are stable in solutions of pH 1-2, with exceptions. At pH 2, copper is 50% complexed and at pH 3.5 is almost completely complexed. Stability constants as reported by Weinstein et al. (71) list Fe^{III} - EDTA complex followed by the complexes with Cu^{II}, Zn^{II}, Fe^{II}, Mn^{II}, Ca^{II}, and Mg^{II}. The calcium disodium salt illustrated has been used in various animal and plant studies.



Chelating Agents in Plant Studies. Chelating agents have been used with some success in increasing iron absorption by plants. Ethylene diamine di-o-hydroxy-phenylacetic acid (EDDHA) was used to study iron absorption by soybeans (72) and sunflowers (73). In both studies it appeared that absorption of iron and EDDHA was in unequal amounts with the iron being released to the roots whereas most of the chelating agent remained in the nutrient solution. Perkins and Purvis (67) used sodium and manganese forms

of EDTA to study the uptake of metals by plants. They found greater increases of extractable Fe^{III} than of Mn^{II}, Ca^{II}, Mg^{II} or K^{I}; thus supporting the stability constant rule. The primary role of these amino polycarboxylic acids seems to be to form water-soluble metal chelates which make the elements available to roots for absorption (71, 74).

EDTA in Mamallian Studies. Some work has been done on the metabolism of EDTA and its effects in mammalian systems. Foreman et al. (75, 76) working with $CaNa_2EDTA$, found that in rats 80-90% of an orally administered dose appeared in the feces within 24 hours. At 6 hours the kidney, liver, muscles, skeleton and GI tract were the only organs containing measurable amounts and these were no more than 0.5%. In a study with human subjects they reported 95% excretion of the C^{14} EDTA portion via urine within 24 hours (77). The $CaNa_2EDTA$ is dissociated by low pH of the stomach; the free EDTA is precipitated and slowly redissolved as it goes through the tract. Johnson and Seven (78) reported that the ferric chelate of EDTA is broken down in the GI tract yielding a black material, probably of sulfides.

Solutions of the disodium salt of EDTA have been used effectively in mobilizing calcium in dairy calves (79). Calcium in blood serum, having a higher stability constant than the sodium, displaces the sodium and forms a soluble, non-ionizable, physiologically unavailable EDTA complex. Lead poisining in calves and horses (80, 81) has been effectively treated with $CaNa_2EDTA$. The lead displaces calcium and forms a non-toxic, non-ionizable complex at physiologic pH.

In work done by Rubin et al. (82), the iron-binding ability of EDTA was compared to that of siderophilin in rabbit serum. Although the ratio of iron-binding ability is 20-25:1, the competition was found to be on an equal basis. Iron added to the serum and bound to one could not be removed by the other.

The sodium salt of EDTA was used by Schanker and Johnson (83) to increase intestinal absorption of certain compounds. The absorption of 2 labeled neutral compounds, mannitol and inulin, was increased from less than 2% without the chelating agent to 7-11% with EDTA. Absorption of a quaternary ammonium compound rose from 2-3% to 11-15% upon the addition of EDTA. They suggested an alteration in permeability of intestinal epithelium as a possible explanation for the increased absorption. Sulfanilic acid absorption increased from 11-14% to 26-32% when EDTA was used. There may be an interference of chelation with calcium or other metal ions in intestinal lumen involved when acids are administered. A final suggestion was that EDTA may act by increasing the size of membrane "pores" or by widening spaces between epithelial cells through the removal of calcium ions.

PROCEDURE

To study the effect of various levels of iron, copper and EDTA on the iron concentration in the liver and spleen, pigs were fed rations with different levels of these three substances. Data on the chemical analysis performed on liver and spleen samples were used to compare the effects of the rations.

Samples Examined in the Experiment

History of the Animals. The Kansas State University Department of Animal Husbandry raised 40 Duroc, Poland China and cross-bred Duroc-Poland China pigs. The 20 barrows and 20 gilts were divided into 5 lots so that sex and breed were equally represented in all lots. From weaning at an average weight of 18.2 kg until slaughter at approximately 91 kg live weight, the pigs were fed rations listed in table 1. Both the pelleted ration and softened water (containing less than 0.03 ppm iron) were available at all times. The pigs were fed in concrete-floored pens. Wooden troughs and galvanized wire were used in the pens to prevent ingestion of copper or iron by the pigs from sources other than the water and ration.

Processing. Animals were starved 24 hours before normal slaughtering procedures were used by the personnel of the university meat laboratory. Liver and spleen samples were removed immediately with a stainless steel knife and were sectioned on a wooden table top. The liver was placed gall bladder side up with the distal end of the lobe toward the meat cutter. The lobes were designated 1, 2 and 3 from left to right as shown in figure 1. The meat cutter removed a 1-cm slice longitudinally from each lobe. A 1-cm slice was cut from each spleen, avoiding the midline of the organ to prevent sampling of the adhering tissue. All samples were wrapped in aluminum foil, labeled with pig number and lobe number or spleen, immediately frozen on a plate freezer at -29°C and then stored at -12 to -18°C.

TABLE 1

Composition of rations

	kg
Lot I: Control[1]	
Sorghum grain (milo)	358.3
Soybean oil meal	43.1
Meat scraps	22.7
Alfalfa meal	22.7
Iodized salt	2.3
B-complex vitamin (Merck 58-A)	0.2
Aurofac 1.8-1.8 (commercial aureomycin and vitamin B_{12})	2.3
$MnSO_4 \cdot H_2O$	0.1
Vitamin A (400,000 IU)	
Lot II: Control + 0.1% $CaNa_2EDTA$	
Lot III: Control + 0.5% $CaNa_2EDTA$	
Lot IV: Low copper and iron[2]	
Ground corn	306.2
Dried skim milk	138.3
Ground limestone	2.3
Iodized salt	2.3
B-complex vitamin (Merck 58-A)	0.2
Aurofac 1.8-1.8 (commercial aureomycin and vitamin B_{12})	2.3
$MnSO_4 \cdot H_2O$	0.1
Vitamin A (400,000 IU)	
Lot V: Control + 53.2 g copper as copper sulfate and 559.8 g iron as ferrous sulfate[3]	

[1]Contains 15 ppm copper and 88 ppm iron

[2]Contains 5 ppm copper and 20 ppm iron

[3]Contains 132 ppm copper and 1320 ppm iron

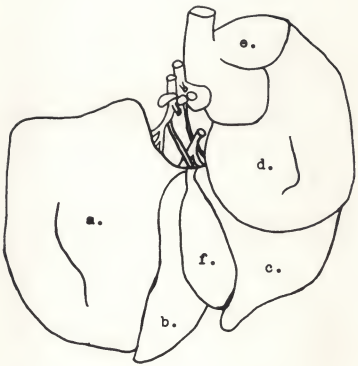


Fig. 1. Diagram of the pig liver showing the order in which the lobes were designated as 1, 2 and 3. a. Left lateral lobe, lobe 1; b., c. Left and right central lobes, respectively, lobe 2; d. Right lateral lobe, lobe 3; e. Caudate lobe, not tested; f. Gall bladder.

Method of Analysis

Four samples (liver lobes 1, 2 and 3 and spleen) were analyzed for each animal. Selection of the order in which the samples were analyzed was accomplished by using a table of random numbers.

Sampling. A 2-cm^2 glassine paper was weighed for each of 6 specimens from each liver or spleen sample. These specimens weighed between 0.08 and 0.12 g. All weighings were on an analytical balance.

Chemical Analysis. All specimens, standards and reagent blanks were subjected to a wet ash digestion. Concentrations of iron were determined by a modification of methods by Smith et al. (84) and Saywell and Cunningham (85). The complete chemical analysis up to reading of absorbancies was done in 15-ml calibrated 52 x 200 mm test tubes. Each time an assay was done, 4 standards and 4 blanks were run with the specimens.

Digestion. One ml of 10-µg iron standard solution was added to each tube designated as a standard and containing a weighed 2-cm^2 glassine paper. One ml of deionized distilled water then was added to each of these tubes. Two ml of deionized distilled water were added to each of the 4 tubes designated as reagent blanks containing a 2-cm^2 weighed glassine paper and to each tube containing a weighed specimen. One ml each of concentrated sulfuric acid and perchloric acid was pipetted into each tube. All tubes were placed in a sandbath on a hot plate (400°C) until the solution was clear and colorless (3-4 hours). When the tubes

were cool, they were removed from the sandbath and covered with plastic film.

Titration. The strongly acid solution was diluted with 1 ml of deionized distilled water, followed by 1 ml hydroxylamine hydrochloride. The solution was titrated with ammonium hydroxide to the bright red of Congo red indicator paper.

Color Development and Reading of Absorbancies. A 0.5-ml portion of 0.15% 4,7-diphenyl-1,10-phenanthroline was added and the solution diluted to the 15-ml volume with deionized distilled water. Ten ml of isoamyl alcohol was then pipetted volumetrically into each tube. Vigorous shaking for 10 seconds resulted in extraction of the color complex by the alcohol. A Beckman DU spectrophotometer with 1-cm silica cells was used to read optical densities (OD) at a wave length of 533 mμ. Each specimen, blank and standard was read against a reference blank of isoamyl alcohol.

Calculation. The following formula was used to calculate the quantity of iron per gram of liver or spleen:

$$\frac{10\ \mu g\ (\text{OD of specimen} - \text{mean OD of blanks})}{\text{Mean OD of standards} - \text{mean OD of blanks}} \div \text{Sample weight} = \mu g\ \text{iron/g}$$

Maintenance of Glassware. With the exception of pipettes and burettes, glassware was washed with detergent and water, rinsed 8 times with tap water, rinsed 8 times with deionized distilled water, inverted on clean paper towels and covered with clean linen towels. When test tubes were dry, they were covered with plastic film and again covered with linen towels. Pipettes

and burettes were rinsed with tap water and placed in a potassium dichromate cleaning solution for at least 15 minutes. They were then rinsed 8 times with tap water and 8 times with deionized distilled water, and placed tip down on clean linen towels. A clean linen towel covered them to protect them from contamination when they were not in use.

Statistical Analyses. Data were tabulated and subjected to analyses of variance. Least significant differences were calculated when appropriate. Sources of variance were as found in table 2.

RESULTS AND DISCUSSION

Liver Iron Concentrations

Mean iron concentrations for liver lobes are shown in table 3 with ranges given in table 5 of the appendix. Lot I, which was the control lot with 15 ppm copper and 88 ppm iron in the ration, had a mean liver iron concentration of 57.2 μg/g of tissue. This value was significantly higher ($P < 0.05$) than Lot III with its mean iron concentration of 35.7 μg/g. These lots received the same levels of copper and iron in the ration but Lot III was fed 0.5% EDTA. Lot II also received copper and iron at the levels of Lots I and III but was fed 0.1% EDTA. The iron concentration of Lot II (39.5 μg/g) was similar to Lot III and not significantly different from Lot I. The EDTA seemed to have a definite depressing effect on liver iron concentrations.

EDTA was shown to enhance absorption of certain substances in mammalian systems (83). If the EDTA was absorbed in the GI

TABLE 2

Sources of variance

Source	df
Liver	
Lots	4
Lobes	2
Animals in same lot	32
Lots x lobes + lobes x animals in same lot	72
Specimens	555
Total	665
Spleen	
Lots	4
Animals in same lot	32
Specimens	185
Total	221

TABLE 3

Mean liver and spleen iron concentrations in pigs fed different levels of iron, copper and EDTA

Lot	Ration	Liver lobes 1	2	3	Liver mean[1]	Specimens	Spleen mean[2]	Specimens
		µg/g	µg/g	µg/g	µg/g	No.	µg/g	No.
I	Control	63.0	52.3	56.3	57.2	126	62.6	42
II	Control + 0.1% EDTA	39.4	40.6	38.5	39.5	144	78.3	48
III	Control + 0.5% EDTA	37.5	36.6	33.1	35.7	108	76.3	36
IV	Low copper and iron	47.9	53.4	47.8	49.7	144	89.4	48
V	High copper and iron	150.6	132.0	119.0	133.9	144	104.3	48
	Lobe mean	67.7	63.0	58.9				

[1] LSD, $P < 0.05$
144, 144 = 19.3
144, 126 = 20.0
144, 108 = 20.9
126, 108 = 21.5

[2] LSD, $P < 0.05$
48, 48 = 9.4
48, 42 = 10.1
48, 36 = 10.5
42, 36 = 10.8

tract, the dietary copper with a higher stability constant (71) would have been complexed rather than the ferrous iron. However, Johnson and Seven (78) found that stability constants could not be used with definite assurance in the body. Also, even if the copper had been preferentially complexed, the iron absorption could still have been normal. Iron absorption evidently was not normal as indicated by the lower mean liver iron concentrations in Lots II and III as compared to the control. Two deaths in Lot III were reportedly caused by anemia and pneumonia. This might be expected if EDTA interferred with iron absorption.

The EDTA could have complexed with the iron and caused excretion of the metal. This chelating agent has been used to mobilize metals in calves and horses (79, 80, 81). As Foreman et al. (76) reported, EDTA passes through the mammalian system without going into RBC and is excreted almost entirely by the kidneys. In rats and humans, 80-95% of an orally administered dose of EDTA was excreted within 24 hours (75, 76). Thus excretion of iron in a complex with EDTA could account for the lower liver iron means in lots fed EDTA.

Lot IV, the low iron (20 ppm) and low copper (5 ppm) ration, had a mean liver iron concentration of 49.7 μg/g which was below that of the control (57.2 μg/g) but not significantly different. A difference in type of rations may have affected utilization of iron (47). Copper enhances the absorption of iron (3, 13, 14, 15, 16, 17, 18, 19), and the more favorable ratio of 1:4 for copper and iron in Lot IV at the low intake may account in part for its favorable iron concentration compared to Lots I, II and

III (1:6). The low iron and copper ration produced liver iron means higher than both Lots II and III (table 3). It would seem that the lower mean liver iron concentrations of Lots II and III were affected by the presence of EDTA.

Lot V, the high iron and copper ration, had a mean liver iron concentration of 133.9 μg/g. This was significantly higher ($P < 0.05$) than the other 4 lots. The copper-iron ratio was 1:10 with 132 ppm copper and 1320 ppm iron. The copper-iron ratios of 1:6 and 1:10 had similar effects on iron and on copper concentrations in the liver, spleen and muscles.[1]

Variation Among Liver Lobes

Table 3 shows the variation among the 3 lobes of the liver. This difference was not significant as indicated in table 6 of the appendix. Readings for each lobe had wide ranges (table 5 of the appendix). When means were calculated and correction made for animal variation, the differences were slight. These data indicated that iron is stored fairly evenly among the lobes of the liver.

Spleen Iron Concentrations

Mean iron concentrations for spleens are shown in table 3 with ranges for individual animals given in table 5 of the appendix. Underwood (3) had reported that the spleen has the highest concentration of iron of any of the organs. However, Josephs (86)

[1]Kainski, Mercedes H., personal communication

found iron concentrations in spleens too varied to include in his study. In the present study, the mean spleen concentrations were higher than the mean liver iron concentrations in all lots except Lot V. While it did not have a spleen iron concentration higher than its liver concentration, Lot V still was significantly higher ($P < 0.05$) than the other 4 lots with a mean of 104.3 µg/g.

The control, Lot I, was significantly lower ($P < 0.05$) than the other 4 lots with a mean spleen iron concentration of 62.6 µg/g. This finding is in contrast to that found in livers where Lots II, III and IV had lower liver iron concentrations than did the control. The presence of EDTA in the ration of Lots II and III may have caused a mobilization of iron such as increased excretion, resulting in iron deficiency. When the iron was depleted in the liver, the spleen made an effort to supply it. Mobilization of splenic iron also was reported when copper was added to an iron-deficient ration (16). This may account for the significantly higher spleen iron concentrations in Lots II and III than in Lot I, although the copper-iron ratios were the same for the 3 lots.

Iron fed with adequate copper is used for Hb synthesis before excesses are stored in tissues (3, 13, 14, 15, 16, 17, 18, 19). The copper-iron ratio of 1:6 in Lot I (62.6 µg/g) was less demanding on the spleen for the mobilization of iron than the ratio of 1:4 in Lot IV (89.4 µg/g). Lot IV was significantly higher ($P < 0.05$) than Lot I.

Iron concentrations in spleens from Lots II and III were similar (78.3 µg/g and 76.3 µg/g). As with livers, there was an

inverse relationship between level of chelating agent fed and concentration of iron found in the tissue. Both lots had significantly lower ($P < 0.05$) spleen iron concentrations than Lot IV which was fed the low copper and iron ration. Again the depression of the iron concentration in the spleen could have been caused by the presence of the chelating agent by the 1:6 copper-iron ratio of the ration.

Animal Variation

Individual animal mean liver and spleen iron concentrations are given in table 4 with the ranges for each animal shown in table 5 of the appendix. Animal variation within lots was very highly significant as indicated by statistical analysis (table 6 of the appendix).

Variation within sexes and breeds and variation within the same sex or breed existed throughout the lots for both livers and spleens. No combination of sex and breed gave consistently high or consistently low values. During the growth period when 3 animals died, this animal variation again was apparent. In Lot I a Poland China barrow died and in Lot III a Duroc gilt and a Poland China gilt died.

The least variation in liver iron concentrations was found in Lots II and III, with means ranging from 31.9 to 49.8 μg/g and 28.6 to 41.4 μg/g, respectively. The means for Lot IV were intermediate from 36.5 to 65.2 μg/g. The greatest differences within lots were reported for Lots I and V, with means from 39.1 to 125.2 μg/g and 100.9 to 184.8 μg/g for differences of

TABLE 4

Mean liver and spleen iron concentrations within lots

Lot	Ration	Animal number	Breed[1]	Sex[2]	Liver	Spleen
					µg/g	µg/g
		80	P	G	125.2	59.7
		81	D	G	49.8	52.2
		82	D	B	46.9	69.0
I	Control	83	P	B	39.1	70.1
		84	D	G	39.9	76.2
		85	X	B	41.9	66.0
		86	X	G	57.8	44.9
		88	D	G	42.6	87.7
		89	P	G	40.7	114.5
		90	X	G	33.9	69.9
		91	D	B	42.6	66.9
II	Control + 0.1% EDTA	92	P	G	49.8	71.9
		93	D	B	38.6	56.4
		94	P	B	35.9	95.4
		95	X	B	31.9	64.0
		96	D	G	34.6	72.2
		97	P	B	34.9	82.6
		98	P	B	38.9	36.3
III	Control + 0.5% EDTA	99	D	G	28.6	107.6
		100	X	B	41.4	69.5
		101	X	B	35.9	89.8
		104	D	G	65.2	83.7
		105	P	B	52.6	150.3
		106	D	B	58.0	89.8
		107	X	B	43.1	81.1
IV	Low iron and copper	108	P	B	41.2	86.6
		109	X	G	36.5	80.9
		110	D	G	44.8	75.2
		111	P	G	55.9	67.8
		112	P	B	149.2	109.6
		113	D	G	109.5	82.1
		114	D	B	118.3	70.0
		115	P	B	137.1	102.6
V	High iron and copper	116	D	G	140.6	112.8
		117	X	B	100.9	121.0
		118	P	G	130.5	126.8
		119	X	G	184.8	109.2

[1] P = Poland China, D = Duroc, X = Duroc-Poland China

[2] G = Gilt, B = Barrow

86.1 µg/g and 83.9 µg/g within lots. Spleens gave opposite results when means were studied. Lots I and V had the narrowest limits of means, from 44.9 to 76.2 µg/g and 70.0 to 126.8 µg/g. Lot IV had the widest limits of means, from 67.8 to 150.3 µg/g, and Lots II and III were intermediate, with means from 56.4 to 114.5 µg/g and 36.3 to 107.6 µg/g of spleen tissue.

Lots II and IV had significantly different spleen iron concentrations (table 3), but 5 animals in Lot II had mean iron concentrations that fell within the range of Lot IV (table 4). Lots II and V also had significantly different spleen iron concentrations (table 3), but 4 animals in Lot II were within the range of concentrations reported for Lot V, and 6 animals from Lot V had concentrations between the low and high found in Lot II. This trend is the same for other lots for both liver and spleen iron concentrations.

Liver and Spleen Iron Concentrations

Liver and spleen iron concentrations were affected by iron, copper and EDTA levels in the rations. The EDTA added at both 0.1 and 0.5% levels had a depressing effect on storage iron in livers. Copper-iron ratios and levels of copper and iron added affected storage. High copper and iron rations caused increases in both liver and spleen iron concentrations. The ratio of 1:4 for copper and iron (Lot IV) was favorable for iron storage in both the liver and spleen, but produced the more noticeable effect in the spleen. Liver iron concentrations seemed to be affected by levels of copper and iron in the ration, whereas

spleen iron concentrations were affected by the ratios of these minerals.

SUMMARY

Forty Poland China, Duroc and crossbred Duroc-Poland China pigs were divided with breed and sex (20 barrows and 20 gilts) equally represented in 5 lots. To study effects of certain levels of iron, copper and EDTA on iron concentrations in the liver and spleen, the pigs were fed various rations. Lot I was fed a control ration containing 15 ppm copper and 88 ppm iron (1:6); Lot II, the control ration plus 0.1% EDTA; Lot III, the control ration plus 0.5% EDTA; Lot IV, low copper (5 ppm) and iron (20 ppm) milk ration with a copper-iron ratio of 1:4; and Lot V, the control ration with copper and ferrous sulfates for a high copper (132 ppm) and iron (1320 ppm) ration with a 1:10 copper-iron ratio.

Pelleted feed and softened water were fed free choice from weaning until approximately 91 kg live weight. Liver and spleen samples were removed immediately after slaughter for chemical analysis. Samples from 3 liver lobes and the spleen were subjected to wet ash digestion and a colorimetric iron assay using 4,7-diphenyl-1,10-phenanthroline.

Livers were affected by levels of iron, copper and EDTA in the ration. The liver iron concentration of animals in Lot III with 0.5% EDTA was significantly lower ($P < 0.05$) than of Lot I. The EDTA lots were similar with 39.5 μg/g for Lot II and 35.7 μg/g for Lot III, but there was an inverse relationship between level

of EDTA fed and iron concentration in the liver. Lot I (57.2 μg/g) was higher than Lot IV (49.7 μg/g) but the difference was not significant. Lot V with a 1:10 ratio had a significantly higher ($P < 0.05$) mean liver iron concentration than the other 4 lots.

Spleen iron concentrations were higher than corresponding liver iron concentrations in all except Lot V. However, Lot V (104.3 μg/g) had a mean spleen iron concentration significantly higher ($P < 0.05$) than the other 4 lots. In spleens, the control (62.6 μg/g) was significantly lower ($P < 0.05$) than all other lots. As with livers, Lots II and III with EDTA were similar with 78.3 μg/g and 76.3 μg/g. Both were significantly lower than Lot IV with 89.4 μg/g. In spleens, the copper-iron ratio of 1:6 in Lots I, II and III seemed to have more of a depressing effect than the chelating agent when these lots were compared with the ratio of 1:4 in Lot IV.

There were no significant differences between iron concentrations in the 3 lobes of the liver, indicating iron probably is stored fairly evenly throughout the liver. Variation within sexes and breeds and within combinations of sexes and breeds were observed throughout the 5 lots. In the liver, the limits of the means were widest in Lots I and V. These lots had the narrowest limits of the means in spleens whereas Lot IV had the widest.

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APPENDIX

TABLE 5

Ranges and mean iron concentrations in livers and spleens of pigs fed different levels of EDTA, iron and copper

Lot and animal number	Lobe 1		Lobe 2		Lobe 3		Spleen	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
Lot I								
80	86.1-303.5	193.1	85.0-119.4	98.6	73.3-102.2	83.9	41.5--72.6	59.7
81	30.7--57.3	44.1	25.4--80.4	51.7	28.1--70.4	53.5	28.8--61.8	52.2
82	31.1--55.7	43.6	35.3--72.2	50.3	22.0--65.5	46.9	47.9--82.7	69.0
83	32.5--66.4	50.6	29.2--43.5	37.8	15.9--42.9	29.0	56.6--78.7	70.1
84	5.7--43.4	22.8	6.3--52.8	31.1	25.7-102.9	65.7	30.8--97.8	76.2
85	27.3--53.7	41.2	11.7--45.2	33.7	32.6--68.2	50.9	55.9--75.1	66.0
86	35.1--59.8	46.0	39.5--72.1	63.2	23.0--90.7	64.3	22.2--68.1	44.9
Mean		63.0		52.3		56.3		62.6
Lot II								
88	27.9--53.5	42.0	37.9--62.3	50.7	20.1--77.7	35.1	41.8-150.0	87.7
89	39.3--65.1	50.5	20.5--52.6	37.7	18.6--44.9	34.0	80.1-161.9	114.5
90	15.9--96.0	37.9	18.8--57.2	38.3	12.0--38.2	25.7	30.2-118.4	69.9
91	40.1--55.3	48.8	31.9--55.6	41.8	25.8--43.8	37.0	43.9--87.6	66.9
92	12.3--58.7	36.3	36.4-104.8	68.5	29.5--98.9	44.5	52.3--99.3	71.9

TABLE 5 (continued)

Lot and animal number	Lobe 1		Lobe 2		Lobe 3		Spleen	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
93	31.3--81.8	45.4	31.3--44.6	38.4	-4.4--63.6	32.0	38.0--66.5	56.4
94	8.1--35.5	29.4	13.8--36.6	26.9	32.0--95.7	51.4	78.6-130.1	95.4
95	14.2--38.9	24.6	6.7--32.1	22.8	31.1--57.3	48.4	45.4--88.7	64.0
Mean		39.4		40.6		38.5		78.3
Lot III								
96	24.8--47.5	36.1	15.7--41.9	28.8	28.2--49.8	39.0	49.6--88.0	72.2
97	28.8-116.9	51.3	1.5--36.9	20.6	17.1--42.4	32.7	75.7--88.3	82.6
98	16.6--86.9	45.1	28.3--38.2	31.9	20.8--59.4	39.9	-2.3--79.4	36.3
99	15.9--39.5	26.9	19.2--46.8	36.4	11.0--29.7	22.5	59.8-137.7	107.6
100	6.8--66.9	40.3	38.8--83.4	61.5	8.6--30.3	22.5	44.7--84.6	69.5
101	18.5--34.2	25.4	17.8--72.1	40.1	25.5--85.1	42.1	66.7-107.8	89.8
Mean		37.5		36.6		33.1		76.3
Lot IV								
104	27.3--73.1	58.6	36.8-230.0	94.7	33.2--52.1	42.5	62.9-103.0	83.7
105	24.5-173.5	64.0	17.5--58.9	43.5	44.5--69.4	50.4	106.5-183.3	150.3
106	39.5--50.7	44.2	67.5--82.4	75.5	20.5--62.7	54.3	73.8-116.5	89.8

TABLE 5 (concluded)

Lot and animal number	Lobe 1		Lobe 2		Lobe 3		Spleen	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
107	27.4--72.3	42.7	27.6--39.4	32.9	28.8--85.9	53.8	24.6-118.0	81.1
108	45.8--67.0	55.5	19.4--30.1	25.3	34.4--53.7	42.6	41.5-133.4	86.6
109	28.2--52.9	38.8	22.8--32.6	28.2	30.6--48.1	42.6	73.0--93.9	80.9
110	4.0--36.0	23.6	36.8--97.6	65.0	37.1--54.4	45.8	59.3--88.6	75.2
111	38.7--64.8	55.4	46.8--75.5	62.0	13.8--75.5	50.1	46.8--97.0	67.8
Mean		47.9		53.4		47.8		89.4
Lot V								
112	81.4-242.7	155.5	120.0-306.1	193.1	65.6-180.5	99.2	88.0-141.1	109.6
113	63.4-159.9	97.4	26.9-176.3	85.7	77.2-243.0	145.4	58.1-113.3	82.1
114	80.3-109.1	97.1	97.3-159.9	134.8	96.9-163.2	123.0	8.9-148.4	70.0
115	59.8-284.1	114.9	69.5-187.7	115.9	105.5-209.3	150.3	73.5-145.8	102.6
116	190.1-420.2	245.2	27.8-125.1	65.6	32.2-175.1	110.8	47.7-148.5	112.8
117	85.9-127.7	105.5	21.3-251.5	100.8	-2.2-184.6	96.4	91.3-141.4	121.0
118	119.1-190.8	149.8	63.0-178.4	127.3	73.6-162.3	114.4	102.6-167.0	126.8
119	133.7-348.0	209.4	124.3-517.4	232.4	59.2-178.3	112.6	78.6-165.5	109.2
Mean		150.6		132.0		119.0		104.3

TABLE 6

Analyses of variance

Source	df	Ms	F	Sig
Liver				
Lots	4	227612.5073	35.33	***
Lobes	2	4522.9642	.70	ns
Animals in same lot	32	6443.3102	5.57	***
Lots x lobes + lobes x animals in same lot	72	4328.0769		
Specimens	555	1156.7575		
Total	665			
Spleen				
Lots	4	10958.5216	18.48	***
Animals in same lot	32	2500.2762	4.22	***
Specimens	185	593.1454		
Total	221			

*** Significant $P < 0.001$

ns Nonsignificant

THE EFFECT OF CERTAIN LEVELS OF IRON, COPPER AND ETHYLENE DIAMINE TETRA-ACETATE IN A PIG RATION ON LIVER AND SPLEEN IRON CONCENTRATIONS

by

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Twenty barrows and 20 gilts representing Poland China, Duroc and crossbred Duroc-Poland China were divided into 5 lots with breed and sex equally represented in each lot. The pigs were fed various rations to study effects of levels of iron, copper and EDTA in rations on iron concentrations in the liver and spleen. Lot I was fed a control ration containing 15 ppm copper and 88 ppm iron with a 1:6 ratio; Lot II, the control ration plus 0.1% EDTA; Lot III, the control ration plus 0.5% EDTA; Lot IV, a low copper (5 ppm) and iron (20 ppm) milk ration with a copper-iron ratio of 1:4; and Lot V, the control ration with copper and ferrous sulfates added for a high copper (132 ppm) and iron (1320 ppm) ration with a 1:10 copper-iron ratio. Pelleted feed and softened water were fed free choice from weaning until slaughter at approximately 91 kg live weight. The liver and spleen were removed immediately after slaughter. Samples from 3 liver lobes and the spleen were subjected to wet ash digestion and a colorimetric iron assay using 4,7-diphenyl-1,10-phenanthroline.

Livers were affected by levels of iron, copper and EDTA. Animals in Lot III (35.7 μg/g) had significantly lower ($P < 0.05$) mean liver iron concentration than animals in Lot I. The EDTA lots were similar with 39.5 μg/g for Lot II and 35.7 μg/g for Lot III, but there was an inverse relationship between level of EDTA fed and iron concentration in the tissue. Lot I (57.2 μg/g) had a higher mean liver iron concentration than Lot IV (49.7 μg/g). The difference was not significant but seemed to be affected by copper and iron levels of the ration. Lot V with a 1:10 ratio

had a significantly higher ($P < 0.05$) mean liver iron concentration than the other 4 lots. The higher levels of copper and iron in the ration seemed to have affected storage in the liver.

Spleen iron concentrations were higher than corresponding liver iron concentrations in all lots except Lot V. However, Lot V (104.3 μg/g) had a mean spleen iron concentration significantly higher ($P < 0.05$) than the other 4 lots. In spleens, Lot I (62.6 μg/g) was significantly lower ($P < 0.05$) than all other lots. As with livers, Lots II and III which received EDTA were similar, with mean spleen iron concentrations of 78.3 μg/g and 76.3 μg/g. Both were significantly lower than Lot IV, with a mean of 89.4 μg/g. The spleen iron concentration seemed to have been affected by the copper-iron ratio and indirectly by the presence of the chelating agent. The ratio of 1:6 for Lots I, II and III had a greater depressing effect than the ratio of 1:4 in the low copper and iron ration of Lot IV.

The 3 liver lobes were found to store iron fairly evenly. Differences between lobes were not significant. Wide animal variation existed in all lots. There were variations within sexes and breeds or any combination of these. With liver iron concentrations the greatest variation was from Lots I and V whereas in the spleen these variations were the least. Spleens in Lot IV had the widest limits of mean iron concentrations.