IRON TOLERANCE IN YOUNG PIGS AS INFLUENCED BY DIETARY VITAMIN E AND SELENIUM

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
KUAN KING KAI
1974

ABSTRACT

IRON TOLERANCE IN YOUNG PIGS AS INFLUENCED BY DIETARY VITAMIN E AND SELENIUM

By

Kuan King Kai

The work presented is an attempt to determine the effects of iron, both oral supplementation and intramuscular injection, in young pigs as influenced by dietary vitamin E and selenium. Three experiments, using a total of 191 pigs, were conducted.

In Experiment 1, 127 pigs, from 17 litters, presumed to be vitamin E - Se deficient, were employed. The animals received their first iron injection at three days of age amounting to 100 mg of iron from iron dextran intramuscularly. They were weaned at 5 weeks of age to a low vitamin E - Se starter diet. Two days after weaning, half of each litter received an intramuscular injection of 34 I.U. of vitamin E + 0.5 mg of Se. Two days after the E - Se injections, half of each of the resulting groups received a second intramuscular injection of 100 mg of iron from iron dextran. No sign of iron toxicity was observed.

In the second experiment, pigs from sows fed a low vitamin E - Se diet were weaned at 5 weeks of age to a fortified grower corn - soy ration. The basal diet was low in vitamin E and Se. Ferrous sulfate ($FeSO_4$, $7H_2O$) and vitamin E as d - α - tocopheryl acetate were added to the basal ration in a 3 x 2 factorial design with 8 pigs per treatment. The diets contained 345, 550 or 850 ppm of iron and either no supplemental vitamin E or 22 I.U. vitamin E/kg of feed. Weight gain, feed intake and feed efficiency were recorded and calculated biweekly. There were no significant differences between treatment groups, during the entire study. There was a significant iron x vitamin E interaction on gain during the growing phase of the trial ($P<.05$).

Hematological analyses conducted at various intervals of the trial revealed no significant treatment differences in hemoglobin, hematocrit and serum iron levels during the entire duration of the trial.

At the end of the study, the experimental animals were slaughtered and samples of muscle, liver and pancreas were collected for iron concentration determination. Here again, it was found that no significant differences occurred between treatment groups in the iron concentration of the tissues taken.

In the third experiment, 16 pigs were used. Eight were weaned from sows which had been fed a low vitamin

E - Se gestation and lactation regime while the other 8 were from sows whose feeds had been supplemented with vitamin E at 22 I.U./kg of feed.

When the animals were about 9 weeks of age, one-half of the presumed vitamin E deficient pigs as well as one-half of the pigs from the vitamin E supplemented sows were given a second iron injection, a massive dose of iron (1000 mg) from iron dextran, intramuscularly. No signs of toxicity were observed following the iron administration.

Blood was collected for hematological studies just before the iron injection as well as at one day and again at one week after treatment for comparative studies.

Serum iron levels had risen dramatically by one day after the injection and were significantly greater than in pigs not receiving the injection ($P<.01$). However, by one week after the injection, serum iron levels had returned to normal control levels leaving no significant treatment differences in serum iron level. Hemoglobin and hematocrit values of iron injected in pigs were not significantly different from those of control animals. Again, there was no evidence of iron toxicity in this study.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Husbandry

1974

ACKNOWLEDGEMENTS

Sincere gratitude is extended to the University of Agriculture, Malaysia, for full financial support, in the form of a scholarship, during the entire period of my graduate studies.

I am deeply grateful to my major professor, Dr. E. R. Miller, who has contributed so much to making my stay at Michigan State University a very pleasant experience. As a teacher, Dr. Miller has earned my heartfelt esteem through his patience, tremendous approachability and his ever-willingness to allow me to share in his deep pool of knowledge and vast personal experiences. I have indeed profited greatly from his guidance and personal examples. I am also indebted to Dr. C. K. Whitehair for his guidance and continued personal interest in my graduate program. Appreciation is also extended to Dr. D. E. Ullrey for serving on my thesis committee.

I am grateful to Dr. Pao Kwen Ku, who has so patiently taught and assisted me in the use of laboratory facilities and in analytical procedures. The assistance of fellow graduate students, laboratory staff and departmental secretaries will always be remembered. I am also thankful

to Miss Kim Miller and Miss Janice Fuller for their skillful typing of this manuscript.

Above all, I wish to thank my wife, Rina, and daughter, Valerie, whose love and encouragement have made this study worthwhile.

VITA

KUAN KING KAI

CANDIDATE FOR THE DEGREE OF

MASTER OF SCIENCE

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I. INTRODUCTION

According to the usual classification of the mineral content in an animal's body, iron is considered as one of the trace elements since it is present in only minute amounts. However, the essentiality of this mineral is beyond question. The reactive part of the hemoproteins, myoglobin and hemoglobin is the iron atom. These hemoproteins serve to store and transport molecular oxygen. In addition, iron is an essential part of various enzyme systems.

The subject of iron deficiency anemia in the pig has been intensively studied and well documented. The baby pig is born with a very low store of iron. Supplementation of the sow's ration with various iron compounds or the parenteral administration of iron preparations during gestation or following parturition does not significantly improve the iron status of the baby pig. The low iron store at birth, together with low iron concentration in the sow's milk plus the rapid growth rate of the pig are factors which predispose it to iron deficiency anemia. Different methods have been proposed to provide a source of available iron to the newborn pig. Allowing the piglets to forage in a trough of soil mixed with iron or the swabbing of the sow's udder with a concentrated iron solution are uncertain and

time-consuming practices which have no place in the present-day swine farm. Oral iron administration is subjected to a host of exogenous factors such as the size of the dose, the state of iron repletion in the body, the presence of phosphates and reducing substances. Numerous workers have shown that intramuscular injection of iron preparations is by far the superior method in terms of promoting maximum hemoglobin synthesis and in increasing weight gain (Browlie, 1955; Gwatkin and L'Ecuyer, 1959; Hydberg et al., 1959; Wahlstrom et al., 1960; Zimmerman et al., 1959; Maner et al., 1959). Today, this method of iron administration to the baby pig for anemia prophylaxis has become standard practice.

In contrast to iron requirement, iron toxicity in the pig is a subject that has not been well studied. In view of the widely emphasized need to provide a supplemental source of iron to the newborn pig, it is appropriate to point out that the indiscriminate use of this mineral is not without risk. There are numerous publications regarding iron poisoning in man - especially children through the accidental ingestion of iron tablets (Wallerstein and Mettier, 1958; Reissmann et al., 1955a,b; Bothwell and Finch, 1962). Much work on the subject has also been done in laboratory animals (Brown et al., 1959; Goldberg et al., 1957; Taylor et al., 1935). Field reports and actual experimental work on iron toxicosis in farm animals are few and far between. In recent years, however, there have been

sporadic field reports of iron toxicity in pigs following either iron prophylaxis or therapy. Initial documented reports were made by Scandinavian workers (Behrens, 1957; Brag, 1958; Nilsson, 1960; Henriksson, 1962). Follow-up studies and investigations by Scandinavian scientists (Lannek et al., 1962; Tollerz and Lannek, 1964; Arpi and Tollerz, 1965) and British researchers (Patterson et al., 1967; Patterson et al., 1969; Patterson et al., 1971) have implicated that vitamin E deficiency would render piglets more susceptible to the toxic effect of iron. High levels of polyunsaturated fatty acid in the gestating sow's diet are believed to aggravate the problem. Studies by Tollerz and Lannek (1964) have indicated that treatment with vitamin E or synthetic antioxidants prior to the administration of iron can afford protection.

The present research project was undertaken to further investigate the toxic effects of iron in vitamin E deficient young swine. An impression has also been gained that selenium deficiency might be another factor which makes the young pig more susceptible to the toxic effects of iron. Selenium has been demonstrated to function as a biological antioxidant. The element is an essential component of glutathione peroxidase (Hoekstra et al., 1973; Scott and Noguchi, 1973). This enzyme is involved in enabling glutathione to perform its role of a biological antioxidant.

II. LITERATURE REVIEW

Iron Metabolism:

In dealing with the subject of iron toxicity, a brief review of iron metabolism seems relevant. Much more about this topic is known in man and laboratory animals than in other species. McCance and Widdowson (1938) were the first to establish that "the amount of iron in the body is regulated by controlled absorption." Following this, the field of iron metabolism and how the body achieves iron balance was intensively studied. Hahn et al. (1943) proposed the "mucosal block" theory and this was later extended by Granick (1954). According to this hypothesis, iron is first reduced to its ferrous form; it is then transported across the mucosal cells bound to ferritin; a second reduction occurs at the plasma membrane, and finally, there is a re-oxidation to the ferric state when it becomes bound to transferrin to be transported in the plasma. Moore (1960) reviewed the mucosal block theory and found it to be inconsistent with regard to the regulation and control of iron transport in the mucosa.

Conrad et al. (1964) put forward a modification of the mucosal block theory. According to these workers, some undefined fraction of the plasma iron serves as a

"messenger" to regulate the uptake of the dietary iron into the mucosal cells where it is bound to protein. The rate at which this iron subsequently enters the circulation is regulated by a balance between the rate at which the epithelial cells are exfoliated and the rate at which the cellular iron enters the plasma.

Dowdle, Schachter and Schenker (1960) have put forward another theory on iron absorption. Using isolated and everted segments of the proximal small intestine of rats, these workers reported that ferrous iron is actively transported; the mechanism is dependent upon oxidative metabolism and the generation of phosphate-bond energy. Jacobs et al. (1966) used a loop of gut with artificial circulation and came up with similar findings. However, Brown and Justus (1958) and Pearson and Reich (1965), also using everted gut loops, were unable to fully confirm the theory that iron is actively transported across the intestinal mucosa via oxidative process. The findingsof Pearson and Reich (1965) that soluble iron did not accumulate in the lumen of the everted gut with increasing iron concentration led them to suggest that iron enters the mucosal cell by diffusion. These workers conceded that it is likely that iron absorption occurs through an active process when the iron concentrations are low, but at higher concentrations, passive diffusion takes over. Charlton et al. (1965) and Wheby (1964) reported that the regulatory activity is lost when iron intake exceeds physiological range. This probably

explains the linear relationships normally cited in that there is always iron absorption at all levels of intake (Bentler et al., 1963; Wallerstein and Mettier, 1958).

Yet another concept on iron absorption which has gained much acceptance is that put forward by Saltman and his associates (Saltman, 1965; Charley et al., 1960; Helbock and Saltman, 1967; Pape et al., 1968; Rubin and Princiotto, 1963). The basis for this hypothesis is directly related to equilibrium binding and chelation phenomena. According to Saltman (1965), Forth et al. (1965) and Ruliffson (1966), both ferric and ferrous iron are available for intestinal transport provided suitable chelates are present. There is no direct dependence on metabolic energy for the accumulation. The amount of iron available from the diet is related to the relative concentrations of those agents which prevent accumulation by forming insoluble or high molecular weight complexes, such as phytic acid and proteins, and low molecular weight substances able to solubilize the iron and permit its absorption. Compounds that increase iron uptake are ascorbic acid, sugars and polyols, amino acids, gluconic acid, α-keto glutaric acid, citric acid, synthetic chelates, alpha-tocopherol, diphenylparaphenylene-diamine. Substances known to inhibit iron uptake are endotoxins, alkalinizing agents, phosphates, oxalates, synthetic chelates and malonic acid. The subject of phytates and iron availability has been studied by various workers. McCance et al. (1943) suggested that phytates

interfere with iron absorption by precipitating iron as insoluble iron phytate, thus making the iron unavailable for absorption. Indian workers (Apte and Venkatachalam, 1962; Sathe and Krishnamurthy, 1953) also demonstrated that phytate considerably reduces the amount of iron available for absorption. In contrast, Harrison and Mellanby (1942) reported that the addition of sodium phytate to the diet of anemic rats fed 0.3 mg iron/day did not appear to inhibit hemoglobin regeneration when compared to control animals. Again, Cowan et al. (1966) compared hemoglobin regeneration in groups of anemic rats fed purified diets containing 10 or 20 ppm iron, in which 45% or 75% of the total phosphorus was replaced with phytate phosphorus. Hemoglobin regeneration was more rapid in the groups receiving 20 ppm iron than in the groups receiving 10 ppm. However, the hemoglobin values indicated that, even at the 10 ppm level, the rate of hemoglobin regeneration was not affected by the presence of either level of dietary phytate. Hence, it was concluded that high levels of phytate have no effect on iron absorption in the rat.

Davis, Luke and Deller (1966) reported the discovery of a gastric inhibitory iron binding protein (gastroferrin), secreted by the gastric mucosa of the stomach. In normal animals and under normal circumstances, this protein complexes with iron and makes the mineral less available to the intestinal mucosa for absorption. This protein appears to be absent in hemochromatosis and low in iron deficiency

anemia. Hence, it has been postulated that this substance forms part of the physiological mechanism controlling iron absorption. In contrast, Murray and Stein (1970a) have studied the long-term effects of achylia gastrica in rats on the absorption of dietary iron. Achylia gastrica was produced in the animals by direct irradiation of the exposed stomach. The treated animals were fed normal diets and it was found that they had less liver iron stores, reduced hemoglobin levels and body weights when compared to normal control animals. The cause was attributed to a defective assimilation of dietary iron as a result of the absence of gastric secretion or acid which is thought to be essential for optimal assimilation of dietary iron by rats.

In another study, Murray and Stein (1970b) confirmed the findings of their previous study and further suggested that defective iron absorption in the irradiated animals can be corrected by the addition of rat or human gastric juice. It is significant to note that intrinsic factors or gastric juice from patients with pernicious anemia did not correct the abnormality. From this, the workers concluded that there must be a factor in the gastric juice necessary for optimal iron absorption in rats when an increased demand for iron was needed.

Mechanism of Absorption of Iron Preparations Administered Intramuscularly:

Beresford, Goldberg and Smith (1957) studied the mode of absorption of a number of polysaccharide complexes

administered intramuscularly into rabbits. In all cases, an acute inflammatory reaction with degenerative changes developed at the injection site. Absorption was mediated partly by this inflammatory reaction with enhancement of lymphatic transport; this was evident from the fact that iron was quickly picked up by the lymph nodes adjacent to the site of injection. Most of the absorption occurred within the first 72 hours; after this period, the iron became rapidly fixed by tissue macrophages and this further contributed to its unavailability. This study also suggested that there is considerable variation in the absorbability of different commercial iron preparations. Miller et al. (1965), in their study on iron retention and ham discoloration in swine with five commercial parenteral iron preparations, agree essentially with this observation.

In another study, Miller et al. (1967) also reported iron injected intramuscularly was quickly picked up and stored by adjacent lymph nodes. This store is then utilized according to body needs for iron. This study concluded that one intramuscular injection of 200 mg iron when piglets are 3 days of age is sufficient to satisfy the body needs for this element and further iron administration later in life could result in muscle discoloration.

Iron Toxicity in Man and Laboratory Animals:

Cases of acute iron toxicity or simply an iron overload in man are well recorded (Wallerstein and Mettier, 1958; Bothwell and Finch, 1962).

Reissmann et al. (1955a) reported that 3-10 g of ferrous sulfate usually prove to be fatal in younger children. Most observers (Swift et al., 1952; Smith et al., 1950; Linquist et al., 1952; Foucar, 1948) attributed death to a local necrotizing action of the iron in the intestinal canal resulting in shock from hemorrhages and fluid loss. Reissmann and Coleman (1955b) disagree with this contention. Working with dogs and rabbits these workers reported that toxicity is due directly to the absorbed iron. The dosage used was between 50 mg to 750 mg iron per kg body weight. The route of administration was either by means of a stomach tube, as an enema in 10% solution or intravenously. Irrespective of the route of administration, none of the animals given 200 mg iron or more per kg body weight survived. Survival time was roughly inversely proportional to the dose administered. The cause of death was reported to be due to a profound metabolic acidosis with blood pH values as low as 6.7. This acidosis was reported to be due mainly to a hydrolyzing effect of ferric iron and in part to an increase in lactic and citric acids. It was suggested that iron in toxic amounts inhibits the enzymes in the Kreb's cycle. Earlier, Racker and Krimsky (1948) had reported that iron shares the property of binding sulfhydryl groups and thus may interfere with certain enzyme systems. These latter workers also observed that glycolysis of tissue homogenates was inhibited by ferrous sulfate.

Bothwell and Finch (1962) reviewed the different types of iron overload in man. Idiopathic hemochromatosis is considered to be associated with excessive absorption of iron as a result of some intrinsic metabolic defect with alteration of the mucosal cell leading to an enhancement of iron uptake. The condition is characterized by liver enlargement with subsequent hepatic insufficiency, pigmentation of the skin and diabetes mellitus; heart failure is a common complication. The condition is believed to be inheritable.

Siderosis amongst South African Bantu is due to an absolute increase in total body iron. This results from the use of iron utensils for cooking and in the preparation of beverages. The quantities present in an average daily diet have been estimated to exceed 100 mg/day and, as the amount of iron retained in the body increases with the size of the dose, iron overload is a common occurrence in the Bantu population. The condition first becomes evident in late adolescence and reaches its peak between the ages of 40 and 50 years. The pathogenesis of the condition initially manifests itself in the liver as parenchymal deposits. With increasing absorption, splenic concentration of iron also rises and often the level in this organ is higher than that in the liver.

Oral iron overload in patients with refractory anemias is due to an increased absorption from the gut. It is associated with an increased, but usually ineffective,

erythropoietic activity and is especially common when therapeutic amounts of iron have been given orally. Parenchymal deposits are usually found and this appears to be related to the fact that the plasma iron level is often persistently raised and the unsaturated iron-binding capacity reduced. The most prominent pathological features are found in the liver; portal cirrhosis and portal tract fibrosis are commonly encountered.

Reticuloendothelial Iron Overload:

Bothwell and Finch (1962) reported that parenteral administration of iron preparations leads initially to an accumulation in the reticuloendothelial system. This is because the iron complexes consist of relatively large molecules that are selectively removed from circulation by the reticuloendothelial system (RES). The RES can tolerate large amounts of iron because it requires very large doses of iron injected into healthy individuals to elicit pathologic changes.

Nissim (1953) reported that subcutaneous injections of "ferric hydroxide ferrous ascorbate" in total dosage of 1 g per kilogram body weight caused hemorrhagic changes in the lungs, patchy parenchymal damage in the liver and adrenal hemorrhages in the guinea pig. Brown et al. (1959) described the effects in dogs when iron was injected parenterally in regular doses. Irrespective of the iron preparations used (iron dextran or saccharated iron oxide)

or by the route of administration (intramuscular, intraperitoneal or intravenous) it was found that dosage of between 2.5 to 3.3 g/kg body weight produced death within 5-10 months. The animals suffered from anorexia, apathy and weight loss, and one dog developed anasarca, edema and chronic leg ulcers. In another study, Brown et al. (1957) reported that dogs which had received intravenously 0.5 to 1.0 g iron per kg body weight at regular intervals lived for 4 to 7 years. Blindness was the only defect during the observed period. After the animals were sacrificed, histological studies revealed that almost no movement of iron out of the reticuloendothelial cells had taken place. Goldberg et al. (1957), using rats and rabbits also found that these species are able to tolerate large doses of iron given intramuscularly. Patterson et al. (1971), however, reported that 47 mg iron per kg body weight given intraperitoneally was fatal to adult rabbits. The cause of death was acute hepatic failure due to iron overload.

Iron Toxicity in Farm Animals:

Nutritional siderosis in beef cattle was reported by Hartley et al. (1959) following the grazing of these animals on pastures containing high levels of iron. The condition was only discovered when the animals were slaughtered; the livers and hepatic and pancreatic lymph nodes were discolored. Bones were osteoporotic and there was hyperplasia of the bone marrow. Biochemical studies of the livers of affected animals revealed very high iron levels.

Effects of high oral iron on dairy cattle were reported by Coup and Campbell (1964). Scouring, loss of weight, reduced milk and butterfat yield were observed when animals were either dosed orally with 30 to 60 g iron per day or when allowed to graze on pastures which were irrigated with water containing high levels of iron.

Lawlor et al. (1965) observed that lambs scoured and performed less well when they were fed rations containing more than 200 ppm supplemental iron. These workers, however, did not discount the possibility of a mineral imbalance in their study.

Iron Toxicity in Swine: Field Reports

Behrens (1957) administered 100 mg iron dextran intramuscularly to 5 one-week-old pigs. Within a few days, all the treated animals exhibited clinical signs such as anorexia, weakness and loss of weight. They died between 8 and 45 days after treatment. Other pigs in the same litter which were not given the iron treatment remained healthy. Postmortem findings in the dead animals included emaciation, edema of the muscles and blackish discoloration of the livers and spleens. Microscopic examination of the livers showed variable amounts of hemosiderin. It was suggested that in the absence of anemia, there is insufficient apoferritin to combine with the iron, which becomes precipitated as hemosiderin.

Brag (1958) described iron toxicity in piglings following a conventional method of anemia prophylaxis. The method

involved the spraying of soil with ferrous sulfate and allowing the piglings to forage in it. Animals reared on concrete were usually 2 weeks old when they were first exposed to this treatment. Signs of illness were observed within a couple of hours after the animals had access to the iron treated soil. As a rule, the whole litter became ill; if there were exceptions, they were usually the smallest and weakest pigs which had been unable to reach the iron soil mixture. Clinical signs included paleness, unsteady gait, convulsion with paddling of the front limbs and difficult breathing; body temperature was usually subnormal and heart rate was accelerated. Within 24 hours the whole litter might be dead. Postmortem findings included paleness of the carcass, waxlike skeletal and cardiac muscles, coagulated milk in the stomach mixed with soil, gastroenteritis and sometimes ulceration of the stomach and intestine. The author reviewed some of the cases of iron poisoning in children and pointed out the similarities in symptoms in both man and pig.

Nilsson (1960) reported the death of 10 piglets 12 hours after receiving 1 to 2 ml of a high molecular weight iron carbohydrate complex intramuscularly. Upon autopsy, the myocardium had a striking tiger stripe pattern with alternating pale and hyperemic parts. Hydropericardium and hydrothorax were also encountered. Microscopically, the myocardial lesion was found to be a focal hydropic

degeneration. Large amounts of glycogen were present in the degenerated muscles.

Blandford and Lodge (1966) reported the case of a 15-week-old pig which died following an injection of an iron dextran preparation. The pig was given 200 mg of the iron preparation at 3 days of age. When it appeared anemic at about 15 weeks of age, a further 300 mg of the same iron preparation was given by subcutaneous route behind the ear. Three days later it was found dead. At autopsy the carcass appeared severely jaundiced and the liver was enlarged. Sections of liver stained with Prussian blue contained only moderate amounts of iron in the Kupffer cells; however, the nuclei of a few small groups of parenchymal cells exhibited either a diffuse Prussian blue reaction or contained discrete Prussian blue positive bodies.

Ueberschär (1966) reported sudden deaths in suckling piglets following administration of iron dextran preparations. At necropsy, severe inflammatory edema and focal muscular necrosis and hemorrhages at the site of injection were found. He concluded that toxic properties of the iron preparation were the cause of these changes.

Experimental Studies of Iron Toxicity in Swine:

Campbell (1961) determined the toxic dose of iron (ferrous sulfate) in piglets 3 to 10 days of age. The iron was administered either by means of a stomach tube or directly into the stomach or duodenum after these organs

were exposed following surgical procedure. Absorption was reported to be rapid both from the stomach and duodenum. It was suggested that when high level of iron is administered directly into the gastrointestinal tract, the mechanism of absorption is by diffusion. The toxic dose was found to be 0.6 g iron per kilogram body weight. Clinical signs and symptoms of toxicity were observed 1 to 3 hours after the toxic dose was given; these included incoordination, marked shivering, hyperpnea and, on handling most of the pigs, tetanic convulsion. Some developed posterior paralysis and, in all cases, there was a profuse diarrhea. Death usually occurred in about 6 hours following the appearance of symptoms. Histopathological studies of animals that had survived up to 24 hours or more had extensive edema along the entire stomach wall. The mucosa showed extensive necrosis and hyperemia. The necrotic tissue contained deposits of yellowish brown to black pigments which gave a positive test for iron with Perl's stain.

It was postulated that the mechanism of toxicity was either due to damage to the gastrointestinal mucosa which led to shock, or directly as a result of high level of plasma iron.

Induced Rickets as a Result of High Density Levels of Iron:

Various researchers working with different species of animals have reported that high levels of dietary iron interfere with phosphorus utilization, especially when the

latter mineral content in the diet is low or just on the borderline of normal requirement (Cox et al., 1931; Deobald and Elvehjem, 1934; Brock and Diamond, 1934). Prolonged excess of dietary iron without at the same time increasing the phosphorus intake can eventually lead to gross phosphorus deficiency with subsequent development of rickets. The condition is believed to be brought about through the precipitation of phosphorus as the insoluble phosphate.

O'Donovan et al. (1963), in their studies with early weaned pigs, reported that pigs fed 3000 ppm iron or less performed well. Pigs fed 4000 ppm iron had slightly depressed serum inorganic phosphorus and phosphorus deficiency rickets was first observed after 6 weeks of the experiment. Pigs which received 5000 ppm iron in their diet had significantly lower serum inorganic phosphorus and femur ash content; rickets with its characteristic symptoms was evident on the 4th week. Symptoms were particularly severe in animals where the levels of serum inorganic phosphorus were lower than 4 mg/100 ml.

This study also demonstrated that the phosphorus level in the diet is important when high levels of iron are present in the diet. Pigs receiving 0.60 or 1.20% phosphorus performed well even though the diet contained up to 5000 ppm iron. However, when animals were fed 0.35% phosphorus and 2500 or 5000 ppm iron, rickets developed.

A separate experiment within the same study indicated that the source of phosphorus was related to the toxic level

of iron in the diet. When the diet contained 5000 ppm iron and the source of phosphorus was either defluorinated rock phosphate, Curacao Island phosphate or commercial dicalcium phosphate, there was a significant reduction in serum inorganic phosphorus values; when monosodium phosphate or dicalcium phosphate (U.S.P.) were used to supply the same level of phosphorus in the diet (0.6%), serum inorganic phosphorus levels were normal.

The work of these investigators are in agreement with those of Hegsted et al. (1949), who reported that rats fed a low phosphorus ration absorbed and consequently stored more iron in the liver than animals fed a similar diet supplemented with phosphorus. Phosphorus (0.8%) was required to prevent marked accumulation of liver iron when the diet contained 0.3% iron as ferric citrate.

Iron Toxicity in Swine Associated with Low Levels of Vitamin E and High Levels of Polyunsaturated Fatty Acids:

Lannek et al. (1962) fed a specially prepared diet, high in polyunsaturated fatty acids (PFA) and low in vitamin E to 3 sows 6 to 27 days before parturition and throughout the lactation period. The sows fed the experimental diet produced milk with about twice the amount of PFA as compared to sows fed a commercial sow ration. The average vitamin E content in the milk fat of treated sows was only about 25% of that in the control sows (average of 11.7 µg/g in treated animals compared to 47.2 µg/g milk fat in control

sows). Seventeen out of a total of 22 piglets from the 3 treated sows were given 150 mg of an organic iron complex intramuscularly when they were between 10-27 days of age. After a few hours or a day following treatment, the animals developed signs of apathy, dyspnea and incoordination. Nine of the 17 treated animals died within $2\frac{1}{2}$ days following treatment. Gross postmortem findings were similar to those described by Brag (1958) and Nilsson (1960): hydropericardium and waxy degeneration of body musculature. There was a significant elevation of plasma glutamic-oxalacetic transaminase (GOT) in affected piglets. In surviving piglets plasma GOT level returned to normal within a week. Two piglets with high plasma GOT on the first day of iron treatment were given α-tocopheryl acetate at 6 mg/kg body weight intramuscularly for 4 consecutive days; on the 5th day, the organic iron complex was again administered. No deaths and no elevation of plasma GOT occurred.

The workers believe that vitamin E exerts an antioxidant effect within the cells. The introduction of iron into tissues containing high PFA and low in vitamin E is thought to speed up the oxidation of PFA and enhance the reactivity of the oxidation products.

In another study, Tollerz and Lannek (1964) obtained vitamin E deficient piglets by the same method as in the previous study. The objective was to demonstrate that vitamin E or synthetic antioxidants can increase the resistance of vitamin E deficient piglets to iron administration.

Each litter was divided into two groups; one group was given only the iron preparation whereas the other group received in addition the substance that was under test. Treatment began when the piglets were 3-8 days old. The iron preparation used was an organic iron complex. It was clear that vitamin E when given 24 hours before the administration of iron protected piglets from death. However, when both vitamin E and iron were given simultaneously, there was no protection. Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) when given together with iron was effective in preventing mortality. Selenium, as sodium selenite, did not give any significant protective action. The results of this study support the hypothesis that at relative over-dosing of iron, vitamin E is active by means of its antioxidant properties.

Arpi and Tollerz (1965) described in detail the post-mortem changes in experimental and spontaneous cases of iron-poisoning in piglets. The experimental piglets were made hypersensitive to both oral and parenteral iron administration by feeding sows a diet deficient in vitamin E. The spontaneous cases were from a farm where deaths occurred following standard prophylactic measures against piglet anemia (100-150 mg of iron from iron dextran intramuscularly).

Postmortem findings in piglets that died following intramuscular injection of iron dextran, iron dextrin or the oral administration of ferrous fumarate were very similar. The skin and muscles were very pale. The muscles

and subcutis around the sites of injection were edematous. The colon, rectum and, to some extent, the small intestine had varying degrees of grayish black discoloration. In some cases there was mild to acute catarrhal enteritis. Slight hydrothorax was reported only in some of the spontaneous cases.

The most striking microscopic finding was a severe waxy muscle degeneration. The pathological condition was characterized by swelling and loss of striation of the muscle fibers and, in some cases, by the total destruction of large areas of muscle fibers. In contrast to the study by Nilsson (1960), who observed a striking tiger stripe pattern of the myocardium, the present workers reported only slight cardiac muscle degeneration in only one of a total of 78 fatalities. Fatty degeneration of muscle fibers and some dystrophic calcification were also reported.

Necropsy findings for piglets poisoned by ferrous sulfate were different from that of the above in two respects. Marked to moderate skeletal muscle degeneration was found only in 3 out of a total of 7 deaths. In all 7 cases, there was a severe acute catarrhal to necrotizing gastroenteritis with necrosis and hemorrhages in the mucous membrane.

Biochemical Studies in Piglets Made Susceptible to the Toxic Effects of Iron:

Patterson et al. (1967a,b), following similar experimental procedures used by Lannek et al. (1962), obtained

litters of pigs susceptible to the toxic effects of iron. Sows on the last 4 weeks of pregnancy were fed a diet deficient in natural antioxidants. Nine piglets from one litter were given 47 mg iron dextran per kg body weight intraperitoneally. Five died within 4 hours of treatment and the others were killed for comparative studies. Chemical analysis and enzymatic assays were determined for muscle, serum and plasma samples. There was a significant increase in the peroxide content of the muscle which was believed to be due to peroxidation of muscle lipid. Raised plasma levels of acid phosphatase are thought to result from lysosomal damage; there was a significant leakage of muscle enzymes such as aspartate aminotransferase into the plasma; more important was the finding that muscle potassium also leaked into the plasma. Death was thought to have resulted from cardiac arrest as a result of hyperkalemia.

In a more thorough study, Patterson et al. (1969) examined in greater detail the biochemical aspects of iron toxicity in susceptible piglets. Two- to three-day-old susceptible piglets were given 47 mg iron dextrose per kg body weight intraperitoneally. Deaths occurred within 5 hours following the iron injection. Pigs that were alive after this period were killed by the intrathecal injection of xylocaine. Blood and muscle were collected for biochemical and histological studies.

The gross and histopathological findings were in general agreement with the description given by Arpi and Tollerz (1965). Coagulative necrosis, loss of striations and, in more severe cases, total destruction of muscle fibers were reported. However, fatty degeneration and dystrophic calcification, which were features described by the latter workers, were absent. An increased amount of stainable iron was detected in the reticuloendothelial cells of the liver. Histochemical studies revealed distribution of succinic dehydrogenase which suggested mitochondrial clumping and probably the disruption of the centers of activity of the mitochondria.

Analysis of muscle and serum iron concentrations showed an absence of a relationship between these criteria and mortality, i.e., there was no critical muscle or serum iron concentration above which the outcome was fatal. There was no significant alteration in muscle dry matter, protein, lipid and nucleic acid contents even in proven cases of myodegeneration. However, in severe cases there was a loss of muscle potassium with corresponding increases in sodium and chloride contents. The raised potassium in the circulation was associated with a significant electrocardiographic response. Fatal cases occurred when the plasma potassium values exceeded about 10 mEq/liter.

Increased iron concentration in the muscle following iron injection brought about an enhanced rate of peroxidation - giving rise to an increased level of tissue

peroxides. This is thought to be responsible for the damage to the lipoprotein envelope of muscle fibers with resultant leakage of cell constituents - potassium and enzymes; the plasma level of aspartate aminotransferase, a muscle enzyme, was raised $2\frac{1}{2}$ to 5 hours following iron injection. There was also an increase in the level of plasma alkaline phosphatase which indicated liver damage. Increased values of acid phosphatase and beta-glucuronidase were thought to be due to early and specific damage to lysosomes. Release of these lysosomal enzymes was thought to initiate the disintegration of the myofibrils. Thus, the underlying mechanism for muscle damage was attributed to an iron-catalyzed peroxidation reaction.

The toxic effect of iron was also manifested by a rise in plasma concentration of glucose. Plasma levels of up to about 500 mg glucose/100 ml were recorded in severe cases. Normal values for three-day-old piglets varied between 115 to 160 mg/100 ml. This increase in plasma potassium is believed to exert an effect on the adrenal medulla and stimulates an adrenalin-sensitive phosphorylase.

The rate of iron absorption following the injection of iron was greater in susceptible piglets than in normal piglets. In normal piglets, maximum muscle iron concentrations were reached 2-3 hours after injection; in susceptible piglets, very much higher maximum muscle values were attained after about one hour. From this it was concluded

that the skeletal muscles of susceptible piglets have an increased affinity for iron.

Analysis of muscle and serum iron concentration prior to iron injection indicated that susceptible piglets had significantly higher values than normal piglets. This coupled with an increased affinity for iron is believed to predispose the muscle to overload following an iron injection.

In yet another study, Patterson et al. (1971) compared the toxic effects of iron in susceptible piglets at 2 and 8 days of age. Clinical manifestations of iron toxicity were found only in the younger age group; 9 out of 16 iron treated animals in this group showed clinical signs such as vomiting and diarrhea, although only 2 of the affected piglets died. Hyperkalemia, though severe in the 2 fatal cases, was not significant when the whole group was taken into consideration. Similarly, plasma aspartate aminotransferase activity was raised in individual piglets but also without significantly affecting the mean value.

The mean muscle iron concentration of the clinically affected group was significantly higher than for the controls; some affected animals which did not have a significant increase in muscle iron concentration were observed to excrete iron stained urine. They believed that, as long as the kidneys were not too severely damaged, and especially in the older piglets, excretion of iron and potassium were protective mechanisms.

Muscle peroxide concentrations were significantly raised only in the 2-day-old piglets that were clinically affected. This finding confirms the hypothesis in an earlier study (Patterson et al., 1969) that peroxidation of muscle lipids is an early step in the acute degeneration of muscle induced by iron dextrose.

III. EXPERIMENTAL PROCEDURE

Animals used in the studies were from either purebred Hampshire or Yorkshire or from crossbred Yorkshire-Hampshire sows which had been fed a low vitamin E - Se or a vitamin E supplemented diet throughout gestation and lactation. The piglets produced by these sows were used in three experiments to examine the tolerance of iron as influenced by the vitamin E and Se status of the animals.

A. General Conduct of Experiments

1. Effect of a second iron injection on vitamin E - Se deficient pigs (Experiment 1)

In this experiment, piglets were obtained from sows which had been fed a low vitamin E - Se ration throughout gestation and lactation. The gestation and lactation diets are shown in Table 1. A total of 127 piglets from 17 litters were used. At three days of age the piglets were given their first intramuscular injection of 100 mg of iron from iron dextran. At 5 weeks of age, these pigs were weaned to a low vitamin E - Se starter ration (Table 1). Feed was provided on an ad libitum basis. Two days after weaning each litter was divided at random into two groups. Pigs in one group received an intramuscular

Table 1. Composition of diets

Ingredient	Gestation %	Lactation %	Starter %	Grower %
Ground shelled corn	85.0	77.5	51.45	78.75
Soybean meal (49%)	11.5	19.0	20.0	18.0
Rolled dehulled oats	-	-	10.0	-
Dried skim milk	-	-	10.0	-
Sucrose	-	-	5.0	-
Ground limestone	1.0	1.0	1.0	1.0
Dicalcium phosphate	1.5	1.5	0.8	1.25
Salt	0.5	0.5	0.5	0.5
MSU VTM premix[a]	0.5	0.5	1.0	0.5
Antibiotic premix[b]	-	-	.25	-
	100.0	100.0	100.0	100.0
Analyses:				
Crude protein, %	13.2	16.1	19.5	16.0
Selenium, ppm	0.04	0.05	0.09	0.05
d-α-tocopherol, mg/kg	7.6	7.4	-[c]	7.5

[a]See Appendix A.

[b]Supplied to the ration 110 ppm chlortetracycline, 110 ppm sulfamethazine and 55 ppm penicillin.

[c]Not analyzed.

injection of 34 I.U. of vitamin E plus 0.5 mg of Se, the other group served as a control. Two days after the vitamin E - Se injection, each group of animals was further divided into two sub-groups. One group of vitamin E - Se injected pigs and one group of non-injected animals were given a second intramuscular injection of 100 mg of iron from iron dextran.

Rectal temperature was taken at 0, 1, 4 and 6 hours after the second iron injection, to determine whether or not there was anaphylactic reaction.

2. Effect of dietary iron supplementation to low vitamin E and vitamin E-supplemented rations of young pigs (Experiment 2)

In this second experiment, 48 pigs from sows on a low vitamin E - Se diet were used. The pigs were weaned at 5 weeks of age. They were allotted randomly by litter and weight into 6 groups of 8 pigs per group. Initially the pigs were kept on aluminum slatted pens until they were approximately $2\frac{1}{2}$ months old, when they were moved to cement slatted pens.

The feed provided following weaning and throughout the experimental period was the grower ration (Table 1). This was a corn-soy diet which contained about 0.05 ppm Se. The basal diet contained 345 ppm Fe. Additions of iron as ferrous sulfate ($FeSO_4$ $7H_2O$) and vitamin E as d - α - tocopheryl acetate to the basal diet were used in a 3x2 factorial design. The diets contained 345, 550 and 850 ppm iron and contained either a low level of vitamin

E or were supplemented with 22 I.U. of vitamin E/kg of feed. Feed was provided on an ad libitum basis and animals were allowed free access to fresh water at all times.

The animals were weighed individually at the beginning of the trial, then weekly or at fortnightly intervals. Following each weighing, feed remaining in the feeders was measured. Feed consumed for the period was recorded and average feed consumption and feed/gain ratios were calculated.

Blood for hematological studies and serum Fe analysis was collected at various intervals during the course of the experiment. Half the number of animals from each group were bled and bleeding was done on the same animals at each subsequent bleeding. Blood was taken from the anterior vena cava by means of a sterilized 18 gauge needle and 20 ml syringe. Samples for hemoglobin (Hb) and hematocrit (Hct) determination were put into acid washed heparinized vials, while samples for serum Fe analysis were placed into acid washed centrifuge tubes.

The length of the experiment was 126 days, after which the pigs were slaughtered. Samples of liver, pancreas and flank muscle were collected from each animal for Fe analysis. Samples were placed in separate polyethylene bags and frozen in the freezer until required for subsequent iron analysis.

3. Effects of a large dose of iron given intramuscularly to young swine deficient in vitamin E and Se (Experiment 3)

Sixteen pigs were used in this study. Eight were weaned from sows fed a low vitamin E - Se gestation and

lactation ration, while the other 8 were from sows whose diet had been supplemented with 22 I.U. of vitamin E/kg of diet. All 16 animals received their first iron injection at three days of age as 100 mg of iron from iron dextran intramuscularly.

At 9 weeks of age, each group of animals was again divided into two sub-groups. One sub-group from each of the two main groups received a second iron injection intramuscularly at a dose of 1000 mg Fe from Fe dextran. The animals were observed for signs of toxicity following the administration of the massive dose of iron.

Blood was collected prior to the administration of the massive dose of iron and then at 1 day and again at 1 week after the iron treatment for hemoglobin, hematocrit, and serum iron determination.

B. Analytical Procedures

1. Hemoglobin

Hemoglobin was determined by the cyanmethemoglobin method of Crosby et al. (1954). A Coleman Junior II spectrophotometer was used for optical density determinations.

2. Hematocrit

Hematocrit was determined by the micro-capillary tube method (McGovern et al., 1955). Blood samples were centrifuged for 5 minutes at 1000 rpm in an International "Hemacrit" centrifuge.

3. Serum collection

Blood samples were collected in acid washed centrifuge test tubes and allowed to coagulate. Following the removal of the clot, samples were centrifuged at 550 g for 15 minutes. The serum was then pipetted into acid washed vials and stored at 5° C.

4. Serum iron

Serum samples were thawed at room temperature and then precipitated with 20% trichloroacetic acid. The mixture was heated in a water bath at 90° C for 15 minutes. It was then removed and kept at room temperature. Upon cooling, the mixture was centrifuged for 5 minutes at 550 g. The supernatant was decanted into acid washed test tubes. Iron concentration was determined by atomic absorption spectrophotometry, using a Jarrell-Ash model 82-516 spectrophotometer. Samples were aspirated into an air-hydrogen flame. An absorption wavelength of 2480.5 Å was used. Samples from pigs which had received the 1000 mg Fe injection were too concentrated and were diluted 1:5 with deionized distilled water prior to iron determination.

5. Determination of iron concentration in feeds

Iron levels in the feeds in Experiment 2 were determined. A wet ash method was used. Depending on the level of iron supplemented, 0.4 to 1.5 g of feed samples from each treatment group were weighed out and placed into separate 250 ml tared Phillips beakers. Sixty milliliters

of concentrated nitric acid were added and digestion was carried out on a hot plate to near dryness and allowed to cool. Seven milliliters of concentrated perchloric acid were added and digestion was allowed to continue again to near dryness. Following cooling, samples were diluted to constant weight with deionized distilled water. Standards were prepared in a similar manner.

Iron concentration was determined by atomic absorption spectrophotometry using a model IL453 Atomic Absorption emission spectrophotometer.* An absorption wavelength of 2486 Å was used.

6. Determination of tissue iron (liver, muscle and pancreas) concentration

A homogenate of the tissue sample was first prepared. Frozen samples of liver, muscle and pancreas were separately cut into thin slices. About 5 g of each tissue sample were used. Deionized distilled water was added to each sample to make up twice the weight of the sample. Homogenization was performed with a Brickmann polytron at high speed for about 1 minute with liver and pancreas samples and for about 2 minutes with muscle samples. In the latter tissue, care was taken to avoid the inclusion of excess fat. The homogenates were poured into polyethylene bags and frozen in the freezer until ready for iron determination.

*Instrumentation Laboratory, Inc., 113 Hartwell Avenue, Lexington, Mass. 02173.

Iron tissue concentrations were again performed by wet ash procedure. The homogenates in the polyethylene bags were thawed with warm water in a beaker. About 5 ml of well mixed samples were pipetted out and placed in separate tared 250 ml Phillips beakers. The beakers containing the samples were then weighed to determine the exact amount of samples used. Digestion was performed as with the feed samples.

Tissue iron concentration was determined by atomic absorption spectrophotometry as mentioned previously for serum-iron determination.

C. Statistical Analysis

The data for experiments 2 and 3 were analyzed for statistical difference by the f-test of Snedecor (1950). Individual treatment values were compared by Duncan's (Bliss, 1967) multiple range test.

IV. RESULTS AND DISCUSSION

Experiment 1

The feeding of a vitamin E - Se deficient diet alone to sows did not produce piglets with reduced tolerance to iron dextran injection. When three days old, piglets from these sows were given an intramuscular injection of 100 mg iron from iron dextran; the animals did not exhibit any signs or symptoms of iron toxicity. Growth was normal and there was no mortality throughout the five week suckling period.

Pigs given a second intramuscular injection of 100 mg iron from the same iron dextran, four days after weaning to a low vitamin E - Se starter diet, also did not develop any reduced iron tolerance. One-half of the experimental pigs had received a vitamin E - Se injection (34 I.U. vitamin E + 0.5 mg Se) two days prior to the iron injection; irrespective of whether the animals had been previously treated with vitamin E - Se injection or not, there was no evidence of iron toxicity.

Rectal temperatures were taken at 0, 1, 4 and 6 hours following the second iron injection, and results are presented in Table 2. Temperatures were all normal with no evidence of anaphylaxis for any of the treatments. There

Table 2. Rectal temperatures, °F, after a second IM iron injection

E - Se inj.[a]	-	-	+	+
Iron inj.[b]	-	+	-	+
Time				
0 hour	102.2	102.3	102.3	102.4
1 hour	103.0	103.1	103.2	103.1
4 hours	103.1	103.4	103.1	102.9
6 hours	102.7	103.0	102.8	103.0

[a]Intramuscular injection of 34 I.U. vitamin E + 0.5 mg Se.

[b]Intramuscular injection of 100 mg iron from iron dextran.

was a slight rise in temperatures during the first one to four hours, but this was believed to be due to excitement during the handling of the animals rather than to any other cause. Six hours after the iron administration, temperatures had all declined or remained constant.

The results of this study appear to be in contrast to those of Lannek et al. (1962) and Patterson et al. (1969). Lannek et al. (1962) reported obtaining piglets with lowered resistance to injectable iron when sows were fed a diet containing 10.4 mg vitamin E per kg of feed for 6 to 27 days during gestation and throughout lactation. When 10- to 27-day-old piglets from these sows were given 150 mg iron from an organic iron complex intramuscularly, signs and symptoms

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of iron toxicity were seen within hours or a day following the injection. There was more than 50% mortality with the treated animals.

Patterson et al. (1969) also obtained piglets that were hypersensitive to the toxic effects of iron by feeding gestating sows a low vitamin E diet. When 3 days old, piglets from these sows were given 47 mg iron from iron dextrose; toxicity and deaths were again reported.

It is important, however, to point out that in both the Lannek et al. (1962) and Patterson et al. (1969) studies, the oil and grain portions of the experimental diets were subjected to oxidizing conditions by heating in a current of air. This increased the peroxide value of the diet and further aggravated the low vitamin E situation. In the present study, no polyunsaturated fatty acids were added to the experimental diets and the peroxide value was not increased by heat treatment. The possibility that the results of this study might have been similar to those of the other workers, had the experimental diets been supplemented with polyunsaturated fats or had the peroxide value been increased, is not ruled out.

Experiment 2:

One pig out of 8 fed the 850 ppm iron ration with no vitamin E supplementation died in the third week of the trial.

Gross postmortem lesions included pale skeletal muscle and a mottled appearance of the cardiac muscles with

alternating areas of hyperemia and paleness. There was a large ulcer at the cardia of the stomach and it was filled with blood. Hydrothorax was also observed.

Histopathological examination revealed extensive necrosis of the myocardium. There was infiltration of mononuclear cells and loss of myocardial fibers. There was some fibrosis in the liver - more than what is expected from an 8-week-old pig.

The gross and microscopic findings are somewhat similar to those described by Brag (1958) in pigs which died following the ingestion of soil mixed with ferrous sulfate solution. The heart lesions correspond with those reported by Nilsson (1960) following the intramuscular injection of iron for anemia prophylaxis. However, in the present case, it is impossible to say with any degree of certainty that the death was the direct result of iron toxicity. Gross and microscopic findings in pigs which died from vitamin E - Se deficiency are also very similar to those described above.

Pig performance data are shown in Table 3. Average daily gains during the growing period tended to increase with increasing levels of iron in pigs fed low vitamin E diets and decreased with increasing levels of iron on high vitamin E diets. Treatment average daily gains did not significantly differ during the finishing period or when the entire study was taken into consideration. Similarly

Table 3. Effect of oral iron and vitamin E on performance

Vit. E, I.U./lb	0			10			±SE[1]	Significance		
Iron, ppm	345	550	850	345	550	850		Iron	Vit. E	Iron x E
No. of animals										
Growing period										
Av. daily gain, lb	1.32[a]	1.39[ab]	1.46[b]	1.48[b]	1.39[ab]	1.30[a]	.046	N.S.	N.S.	P<.05
Av. daily feed, lb	3.75	3.77	3.81	3.77	3.53	3.84				
Gain/feed	0.35	0.37	0.38	0.39	0.39	0.34				
Finishing period										
Av. daily gain, lb	1.48	1.37	1.54	1.41	1.59	1.52	.06	N.S.	N.S.	N.S.
Av. daily feed, lb	5.09	4.83	5.18	4.85	5.29	4.94				
Gain/feed	0.29	0.28	0.30	0.29	0.30	0.31				
Overall										
Av. daily gain, lb	1.39	1.39	1.50	1.46	1.46	1.39	.09	N.S.	N.S.	N.S.
Av. daily feed, lb	4.25	4.19	4.34	4.19	4.21	4.28				
Gain/feed	0.33	0.33	0.35	0.35	0.35	0.32				

[1]Standard error of the mean.

N.S. = not significant.

[ab]Values in the same row with different superscripts differ significantly (P<.05).

there were only small treatment differences in daily feed consumption and gain/feed ratios.

The iron-vitamin E interaction in average daily gains during the growing period was rather unexpected. The dietary iron requirement for pigs at this age is far below the levels that were used in this study. The optimum iron requirements for the growing pig have been given at between 60 to 125 ppm (Matrone et al., 1960; Ullrey et al., 1960). These values vary depending on the animal's iron store, the type of ration fed, the phosphorus level in the diet, and other factors. The National Research Council (1968) has considered all these factors and has established 80 ppm as a level of iron required by the pig. Increasing the level of dietary iron above optimal requirement does not normally bring about better performance. The term "optimal" is used here in a relative sense; optimal requirement for one individual might not be so for another.

The decrease in daily gains during the growing period with increasing levels of iron on the high vitamin E diet was also unexpected. When the levels of iron are considered, the highest level used (850 ppm) is nowhere near the level which has been found to bring about a depression in growth rate. O'Donovan et al. (1963) reported that young pigs receiving up to 3000 ppm iron performed well. It was only when the diet contained 4000 ppm iron that rate of gain became significantly reduced. A similar observation was made by Furugouri (1972). When 60-day-old pigs

were fed up to 3102 ppm iron, there was no significant difference in weight gain and feed consumption. When animals were given 5102 ppm dietary iron, both feed intake and rate of gain were depressed. One possible explanation of the results of the present study is that a high level of vitamin E is somehow incompatible with a high iron level. The exact mode of incompatibility (if it exists) cannot be explained without further investigation.

There was a significant ($P<.05$) iron x vitamin E interaction on gain during the growing period of the study. Here again, the indication suggests that high vitamin E is not compatible with high iron.

Hematological data for this experiment are presented in Table 4. There were no significant treatment differences in hemoglobin, hematocrit or serum iron. Hematological values would all be considered normal and there was no evidence of iron toxicity with increasing levels of iron. These results suggest that once the animal's iron requirement is met, further availability of the element would not contribute to greater hemoglobin synthesis or an increase in the number or size of the erythrocytes. Again, as the serum iron concentrations were not affected by increasing the dietary iron levels, the excess iron must either be excreted and/or stored. If the surplus iron or part of it is excreted, there must be some active mechanism in the normal gastrointestinal tract which brings about this process.

Table 4. Effect of oral iron and vitamin E on hematology

Table 4. Effect of oral iron and vitamin E on hematology

Vit. E, I.U./lb	0			10			±SE[1]	Significance		
Iron, ppm	345	550	850	345	550	850		Iron	Vit. E	Iron x E
No. of animals	4	4	4	4	4	4				
Hemoglobin, g/100 ml										
4 weeks	11.20	9.86	10.59	9.34	11.00	10.17	.57	N.S.	N.S.	N.S.
10 weeks	10.41	11.03	11.55	10.96	11.44	12.14	.57	N.S.	N.S.	N.S.
14 weeks	10.65	10.72	11.44	10.42	10.02	9.97	.67	N.S.	N.S.	N.S.
17 weeks	11.44	12.07	10.80	9.80	10.74	10.66	.52	N.S.	N.S.	N.S.
Hematocrit, %										
4 weeks	35.3	36.3	36.5	34.5	35.4	36.1	1.23	N.S.	N.S.	N.S.
10 weeks	36.5	38.3	38.8	34.7	37.7	37.1	1.96	N.S.	N.S.	N.S.
14 weeks	38.2	38.4	39.2	37.4	36.7	34.0	1.71	N.S.	N.S.	N.S.
17 weeks	16.6	39.8	34.4	33.3	38.0	36.6	1.80	N.S.	N.S.	N.S.
Serum Fe, µg/100 ml										
4 weeks	196.3	134.1	202.8	214.8	191.1	155.3	29.59	N.S.	N.S.	N.S.
10 weeks	150.5	168.4	168.3	168.1	180.0	163.5	18.06	N.S.	N.S.	N.S.
14 weeks	259.8	277.6	269.4	230.2	218.4	230.5	27.53	N.S.	N.S.	N.S.
17 weeks	134.9	128.2	143.7	109.7	157.5	127.5	14.53	N.S.	N.S.	N.S.

[1]Standard error of the mean.

N.S. = not significant.

The results of this experiment agree with those of O'Donovan et al. (1963), Standish et al. (1971) and Harmon et al. (1970), but disagree with those of Koong et al. (1970) and Furugouri (1972). Koong et al. (1970) reported that when young calves were fed increasing levels of iron (100 to 2000 ppm), blood hemoglobin and serum iron values increased significantly. Furugouri (1972) also reported that hemoglobin concentration increased linearly with increasing levels of iron and suggested that absorption of excess iron stimulates marrow activity.

Tissue iron concentrations are shown in Table 5. Liver iron concentrations were generally higher than normal. This was not unexpected as the lowest level of dietary iron fed was 345 ppm - about 250% more than the level that is normally found in the feed. Liver iron levels tended to increase with increasing levels of iron in pigs fed the high vitamin E diets. However, these increases are not statistically significant. Furugouri (1972) reported that when higher levels of dietary iron were used, there was a linear increase in the total liver iron stores, which indicates that the iron storage capacity of the liver is relatively high. Furugouri (1972) believes that the liver iron storage capacity alleviates iron toxicity from excess in dietary iron. In man, it has been reported (Bothwell and Finch, 1962) that with excessive dietary iron over a prolonged period of time there is an increased accumulation of the element in the liver. However, it takes a period of many

Table 5. Tissue iron concentration in Experiment 2

Vit. E, I.U./lb	0			10			±SE[1]	Significance		
Iron, ppm	345	550	850	345	550	850		Iron	Vit. E	Iron x E
No. of animals	6	6	6	6	6	6				
Liver, ppm iron	194.4	186.3	228.7	187.3	207.2	382.8	26.17	N.S.	N.S.	N.S.
Muscle, ppm iron	12.2	11.4	11.5	11.5	11.0	14.3	1.14	N.S.	N.S.	N.S.
Pancreas, ppm iron	14.4	16.5	14.4	12.4	15.1	16.3	2.16	N.S.	N.S.	N.S.

[1]Standard error of the mean.

N.S. = not significant.

years before clinical signs are manifested. In the pig, this is not likely to constitute a practical problem, as the animal's life is usually terminated before the iron accumulated in the liver can give rise to clinical problems. On the other hand, it must be remembered that excess dietary iron can interfere with phosphorus utilization and bring about an induced rickets (O'Donovan, 1963).

Muscle and pancreas iron concentrations in pigs fed high iron levels were not significantly different between treatment groups.

Experiment 3:

Hematological data for Experiment 3 are presented in Table 6. There were no significant treatment differences in hemoglobin or hematocrit levels at 0, 1 day and 1 week after the intramuscular injection of 1000 mg iron from iron dextran. Serum iron levels, however, rose dramatically and, the day following the injection, were significantly ($P<.01$) greater than in pigs not receiving the injection. Further, iron treated pigs from sows receiving the vitamin E supplemented diet had significantly ($P<.01$) greater serum iron concentrations one day after injection when compared to pigs from sows fed the low vitamin E diets. However, one week after the iron injection, serum iron levels of treated pigs had returned to the baseline control levels, leaving no treatment differences in serum iron level between the control and treated animals. Observations on behavior,

Table 6. Effect of vitamin E and IM injection of 1000 mg Fe on hematology

Vit. E, I.U./lb	0		10		±SE[1]	Significance		
Iron injection[a]	-	+	-	+		Iron	E	Iron x E
No. of animals	4	4	4	4				
Initial								
Hemoglobin, g/100 ml	11.10	9.53	10.68	10.75	.63	N.S.	N.S.	N.S.
Hematocrit, %	37.50	36.10	35.95	36.00	1.19	N.S.	N.S.	N.S.
Serum Fe, µg/100 ml	165.48	204.98	192.35	150.70	27.59	N.S.	N.S.	N.S.
1 day								
Hemoglobin, g/100 ml	11.30	9.68	9.90	10.10	.50	N.S.	N.S.	N.S.
Hematocrit, %	36.18[b]	33.00[c]	30.03[b]	33.93[d]	2.21	N.S.	N.S.	N.S.
Serum Fe, µg/100 ml	235.03[b]	3478.13[c]	205.20[b]	3920.05[d]	240.26	P .01	N.S.	N.S.
1 week								
Hemoglobin, g/100 ml	11.83	11.38	11.60	10.50	.32	N.S.	N.S.	N.S.
Hematocrit, %	34.28	35.63	35.18	29.83	1.96	N.S.	N.S.	N.S.
Serum Fe, µg/100 ml	157.03	188.05	206.55	188.05	18.76	N.S.	N.S.	N.S.

[1]Standard error of the mean.

N.S. = not significant.

[a]Intramuscular injection of 1000 mg of iron from iron dextran at 4th week of the study.

[bcd]Values in the same row with different superscripts are significantly (P<.01) different.

performance and hematological data provided no evidence of iron toxicity.

This study clearly suggests the tremendous tolerance of iron by the young pig and demonstrates the innocuous nature of iron dextran. This good tolerance was exhibited both by the pigs from sows fed vitamin E supplemented diets as well as by the presumed vitamin E deficient animals. The fact that the serum iron levels in the pigs from sows fed vitamin E supplemented diets were significantly higher than those from sows with low E diets might be because vitamin E enhances iron uptake when iron is given by the intramuscular route. The high levels of iron in the serum did not seem to be harmful to the animals in this short term study. Iron is known for its peroxidation action. This being so, one would expect an increase in the rate of peroxidation to occur in the presumed vitamin E deficient animals. Lipid peroxide formation could then bring about hemolysis. However, normal hemoglobin and hematocrit values in this study suggest that this has not occurred. Again, the short term study conducted here does not permit any real conclusion to be made as far as this area is concerned.

V. SUMMARY AND CONCLUSIONS

Three experiments were conducted to study the iron tolerance of young pigs as influenced by dietary vitamin E and selenium. Intramuscular administration of up to 1000 mg of iron from iron dextran or oral administration of up to 850 ppm of iron from ferrous sulfate in the diet for 126 days warranted the following conclusions:

1. Sows fed low vitamin E - Se corn-soy fortified diets during gestation and lactation did not produce piglets with a reduced tolerance to the standard dose of 100 mg iron from iron dextran when given at three days of age.

2. Iron toxicosis was not produced when young pigs weaned at five weeks from sows on a low vitamin E - Se diet were given a second iron-dextran injection of 100 mg iron.

3. Pigs weaned from sows on low vitamin E - Se diets and kept on a low E - Se corn-soy fortified diet up to market weight did not exhibit any evidence of iron toxicity when their feed contained up to 850 ppm iron.

4. There was an increase in liver iron storage with excess dietary iron. However, this increase was not linear and, with further additional dietary iron levels, there was no significant proportional increase in liver storage.

5. Hemoglobin, hematocrit and serum iron levels were not significantly affected by dietary iron levels above the normal requirements.

6. An intramuscular injection of 1000 mg iron from iron dextran given to nine-week-old pigs produced a tremendous increase in serum iron levels; pigs on the vitamin E supplemented diets had significantly higher serum iron levels than those on the low vitamin E diet.

7. High serum iron levels did not produce any noticeable ill effect on the animals.

8. Hemoglobin and hematocrit values were not affected by a large dose of iron dextran given intramuscularly when the iron requirement of the pig was already met.

9. Pigs can tolerate intramuscularly administered iron dextran with a high degree of safety.

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APPENDIX

APPENDIX A

Table A-1. Vitamin-Trace Mineral premix

Nutrient	Amount in 10 lbs of premix
Vitamin A, million	3.0 I.U.
Vitamin D_2, million	0.6 I.U.
Riboflavin	3.0 g
Nicotinic acid	16.0 g
d-pantothenic acid	12.0 g
Choline chloride	100.0 g
Vitamin B_{12}	18.0 mg
Zinc	68.0 g
Manganese	34.0 g
Iodine	2.5 g
Copper	9.0 g
Iron	54.0 g
Antioxidant[a]	45.0 g
Carrier (ground yellow corn)	to bring to 10 lbs

[a]Butylated hydroxyanisole (BHA) and/or butylated hydroxytoluene (BHT).

Table A-2. Performance data, Experiment 2, low vitamin E with 345 ppm iron

Period	Initial	1 wk	3 wk	5 wk	7 wk	9 wk	11 wk	13 wk	15 wk	17 wk	18 wk
Pig no.						Weight, lbs					
108-9	31	41	56	66	-	-	-	-	-	-	-
111-2	28	35	53	71	96	120	146	171	194	222	227
Y2-5	25	35	50	64	88	93	118	138	153	178	182
110-3	25	36	55	70	93	111	123	142	156	172	173
H2-1	27	34	50	67	89	110	134	137	172	192	204
205-2	27	34	52	68	90	116	144	170	199	232	241
Y1-6	30	39	57	73	93	112	123	147	166	190	198
106-7	23	32	49	63	85	105	122	146	166	180	194
Av. daily gain, lb		1.25	1.22	1.16	1.25	1.30	1.32	1.34	1.35	1.40	1.38
Feed cons. to date, lb		126	473	812	1280	1668	2150	2617	3075	3622	2897
Av. daily feed, lb		2.25	2.81	2.90	3.32	3.50	3.75	3.89	3.99	4.17	4.25
Feed/gain		1.80	2.29	2.49	2.64	2.70	2.83	2.90	2.97	2.98	3.07

Table A-3. Performance data, Experiment 2, high vitamin E with 345 ppm iron

Period	Initial	1 wk	3 wk	5 wk	7 wk	9 wk	11 wk	13 wk	15 wk	17 wk	18 wk
Pig no.						Weight, lbs					
111-8	29	38	51	64	84	103	129	133	159	179	188
205-1	28	36	53	68	90	113	145	167	179	205	211
110-9	30	41	62	69	100	124	146	165	176	196	206
121-3	27	35	51	67	94	118	147	163	178	200	216
205-8	23	33	48	63	81	104	131	156	173	186	196
207-6	26	34	50	66	87	112	138	158	185	208	221
Y1-2	29	39	59	75	99	121	145	168	182	211	213
106-6	29	37	56	74	99	121	150	174	202	229	234
Av. daily gain, lb		1.28	1.18	1.20	1.28	1.38	1.48	1.46	1.44	1.46	1.45
Feed cons. to date, lb		124	475	827	1332	1759	2316	2809	3286	3879	4216
Av. daily feed, lb		2.21	2.82	2.95	3.33	3.49	3.76	3.88	3.91	4.07	4.18
Feed/gain		1.72	2.38	2.46	2.60	2.53	2.55	2.64	2.71	2.78	2.88

Table A-4. Performance data, Experiment 2, low vitamin E with 550 ppm iron

Period	Initial	1 wk	3 wk	5 wk	7 wk	9 wk	11 wk	13 wk	15 wk	17 wk	18 wk
Pig no.						Weight, lbs					
108-1	28	37	51	67	87	110	138	166	190	212	205
108-11	25	38	47	61	80	101	124	148	173	202	205
Y2-6	25	35	53	72	93	115	144	171	189	196	218
110-4	27	37	54	70	91	110	133	158	181	206	212
205-5	26	33	50	66	90	112	137	157	175	189	194
H3-4	27	34	50	65	86	104	127	146	160	173	187
Y1-1	25	34	52	68	81	103	130	155	177	204	208
102-10	25	34	48	64	83	104	123	137	143	162	165
Av. daily gain, lb		1.32	1.17	1.16	1.20	1.29	1.38	1.41	1.40	1.40	1.38
Feed cons. to date, lb		134	479	838	1332	1760	2327	2894	3352	3929	4216
Av. daily feed, lb		2.39	2.85	2.98	3.33	3.49	3.78	3.98	3.99	4.12	4.18
Feed/gain		1.81	2.43	2.59	2.76	2.70	2.74	2.81	2.84	2.94	3.04

Table A-5. Performance data, Experiment 2, high vitamin E with 550 ppm iron

Period	Initial	1 wk	3 wk	5 wk	7 wk	9 wk	11 wk	13 wk	15 wk	17 wk	18 wk
Pig no.						Weight, lbs					
108-5	30	37	55	73	97	121	148	174	192	209	242
108-12	29	35	50	64	79	98	119	141	216	176	187
203-1	22	27	42	61	90	118	154	186	160	244	252
110-11	30	41	60	77	100	118	138	163	177	197	205
119-5	26	31	43	59	81	103	135	169	193	204	216
202-4	25	33	48	60	79	103	132	160	176	196	210
102-8	24	31	44	60	80	98	120	142	165	189	192
106-12	23	31	47	59	77	98	117	135	152	166	177
Av. daily gain, lb		1.01	1.07	1.09	1.18	1.28	1.39	1.46	1.45	1.44	1.46
Feed cons. to date, lb		103	407	744	1216	1631	2172	2802	3338	3922	4248
Av. daily feed, lb		1.83	2.42	2.65	3.04	3.24	3.53	3.85	3.97	4.12	4.21
Feed/gain		1.80	2.26	2.45	2.57	2.52	2.54	2.64	2.73	2.86	2.89

Table A-6. **Performance data, Experiment 2, low vitamin E with 850 ppm iron**

Period	Initial	1 wk	3 wk	5 wk	7 wk	9 wk	11 wk	13 wk	15 wk	17 wk	18 wk
Pig no.						Weight, lbs					
108-4	26	31	Died	3/12	(34# -	ulcer)					
Y2-4	31	41	63	84	110	133	154	185	212	244	248
110-10	31	42	60	80	96	116	138	156	172	203	196
Y3-9	21	29	45	60	83	103	122	155	179	207	214
202-3	28	36	55	74	96	123	153	172	205	224	237
207-5	23	30	48	62	83	106	128	150	172	196	208
102-1	25	33	47	65	85	110	134	157	176	182	188
121-1	26	36	53	71	94	116	145	171	192	211	125
Av. daily gain, lb		1.20	1.24	1.12	1.30	1.40	1.45	1.50	1.52	1.53	1.49
Feed cons. to date, lb		110	466	784	1254	1635	2084	2591	3091	3591	3864
Av. daily feed, lb		1.20	3.03	3.11	3.51	3.65	3.82	4.02	4.17	4.28	4.35
Feed/gain		1.64	2.44	2.78	2.69	2.61	2.62	2.68	2.74	2.79	2.91

Table A-7. Performance data, Experiment 2, high vitamin E with 850 ppm iron

Period	Initial	1 wk	3 wk	5 wk	7 wk	9 wk	11 wk	13 wk	15 wk	17 wk	18 wk
Pig no.						Weight, lbs					
108-6	32	42	62	82	109	133	145	178	202	222	235
110-1	29	40	61	83	109	133	147	177	186	206	212
110-8	28	36	52	63	87	106	120	156	162	186	184
234-9	24	31	45	60	83	104	120	144	169	187	206
H1-1	23	30	48	61	84	108	122	144	170	194	202
Y1-4	29	40	58	69	95	116	125	152	171	186	194
102-12	25	32	50	67	88	107	120	146	162	182	189
121-6	28	35	50	67	87	106	122	146	166	188	195
Av. daily gain, lb		1.21	1.24	1.17	1.28	1.36	1.29	1.40	1.38	1.39	1.38
Feed cons. to date, lb		126	488	843	1373	1861	2366	2910	3411	4003	4301
Av. daily feed, lb		1.60	2.90	3.01	3.43	3.69	3.84	4.00	4.06	4.20	4.27
Feed/gain		1.46	2.33	2.57	2.67	2.71	2.98	2.86	2.94	3.02	3.09

Table A-8. Hematological data, Experiment 2

Treatment	Pig no.	Hemoglobin g/100 ml				Hematocrit %				Serum iron µg/100 ml			
		Ini.	10 wk	14 wk	17 wk	Ini.	10wk	14wk	17wk	Ini.	10 wk	14 wk	17 wk
0 I.U. vit. E	111-2	11.51	12.02	10.48	10.66	38.1	39.1	37.9	34.2	148.5	151.0	227.1	154.7
345 ppm Fe	Y2-5	11.46	11.00	11.08	11.47	35.3	36.3	37.4	36.8	168.1	133.7	178.1	146.0
	205-2	10.31	9.42	8.95	11.17	32.2	35.1	37.8	39.1	200.1	104.9	260.0	113.9
	Y1-6	11.51	9.21	12.10	12.45	35.6	35.6	39.5	36.5	262.5	212.5	273.9	125.1
10 I.U. vit. E	111-2	9.04	9.84	9.46	13.30	33.8	30.5	35.9	39.2	182.0	165.5	207.4	93.8
345 ppm Fe	205-1	10.10	11.38	12.36	12.37	33.8	36.7	37.9	38.7	175.3	168.4	238.9	73.8
	207-6	8.23	10.78	9.38	12.37	33.2	33.9	38.5	38.7	124.6	130.8	224.3	125.1
	Y1-2	9.97	11.85	10.48	12.45	37.2	37.8	37.2	38.2	377.2	207.6	250.2	146.6
0 I.U. vit. E	Y2-6	9.04	9.80	10.31	13.21	36.0	37.7	38.9	40.0	169.9	180.0	223.1	140.1
550 ppm Fe	205-5	8.99	9.80	10.40	11.72	35.0	37.1	38.0	39.2	182.0	180.0	267.1	76.6
	H3-4	9.89	12.62	10.57	12.79	39.2	41.9	40.1	48.0	77.8	174.2	242.3	154.8
	110-4	11.51	11.90	11.59	10.57	35.1	36.5	36.8	32.2	106.8	139.4	277.9	141.2
10 I.U. vit. E	108-5	12.27	13.43	12.79	12.36	37.2	42.8	41.8	33.4	200.1	174.2	222.5	176.5
550 ppm Fe	110-11	12.36	11.59	9.89	10.66	37.2	37.0	37.8	38.4	192.0	177.1	220.9	157.5
	202-4	10.70	10.31	9.12	10.66	33.9	37.7	37.3	40.5	184.0	185.9	197.3	171.0
0 I.U. vit. E	Y2-4	10.19	12.15	9.89	9.00	38.4	40.6	41.6	33.4	181.2	146.4	286.2	135.8
850 ppm Fe	110-10	10.36	11.46	13.55	11.00	39.1	40.1	41.7	29.5	250.0	152.1	210.9	119.7
	Y3-9	11.12	11.12	11.06	12.28	34.9	36.7	35.3	37.1	245.2	180.1	262.3	119.7
	102-1	10.70	11.46	11.25	11.00	33.6	37.8	38.2	37.4	134.9	194.7	318.4	199.5
10 I.U. vit. E	110-8	12.27	12.79	10.40	11.47	39.5	41.2	37.9	38.5	135.2	136.5	236.6	149.3
850 ppm Fe	234-9	9.29	11.08	10.23	9.38	32.1	36.2	34.9	37.8	176.3	99.2	280.4	122.6
	H1-1	9.97	12.66	10.14	10.23	39.2	43.8	37.5	32.5	153.9	173.7	133.1	99.5
	102-12	9.16	12.02	9.12	11.59	33.7	27.2	25.8	37.4	155.1	244.6	271.9	138.6

Table A-9. Tissue iron levels, Experiment 2

Treatment	Pig No.	ppm		
		Liver	Muscle	Pancreas
0 I.U. vit. E	111-2	155.9	8.7	12.8
345 ppm Fe	205-2	160.4	9.7	9.2
	110-3	247.1	17.5	20.2
	106-7	266.9	12.0	16.2
	H2-1	141.9	13.3	13.8
	Y1-6	194.4	12.2	14.4
10 I.U. vit. E	205-1	103.4	15.6	8.5
345 ppm Fe	207-6	201.6	7.4	15.0
	Y1-2	268.3	11.8	14.3
	121-3	125.2	9.3	11.5
	106-6	281.1	11.1	12.9
	205-8	144.2	14.0	11.9
0 I.U. vit. E	205-5	194.3	10.8	11.3
550 ppm Fe	H3-4	159.0	11.3	11.4
	Y1-1	223.4	8.8	13.0
	108-1	145.2	11.3	37.4
	102-10	209.8	14.6	9.4
	Y2-6	186.3	11.4	16.5
10 I.U. vit. E	203-1	127.6	10.0	11.1
550 ppm Fe	202-4	147.i	7.7	11.9
	108-12	202.6	10.7	18.3
	106-12	299.2	12.0	15.1
	102-8	258.5	14.4	18.9
	110-11	207.2	11.0	15.1
0 I.U. vit. E	110-10	253.5	11.3	22.9
850 ppm Fe	102-1	258.4	14.3	10.1
	121-1	206.6	8.9	16.4
	207-5	268.9	11.9	11.8
	202-3	155.9	10.9	10.9
	Y3-9	228.7	11.5	14.4
10 I.U. vit. E	110-8	274.9	20.0	13.3
850 ppm Fe	234-9	138.2	8.7	16.4
	H1-1	135.4	10.5	15.0
	102-12	303.3	15.2	19.5
	121-6	221.3	14.6	16.0
	110-1	382.8	16.5	17.7

Table A-10. Hematological data, Experiment 3

Treatment	Pig no.	Hemoglobin, g/100 ml			Hematocrit, %			Serum iron, µg/100 ml		
		Ini.	1 day	1 wk	Ini.	1 day	1 wk	Ini.	1 day	1 wk
0 I.U. vit. E	110-7	11.0	9.2	9.9	34.5	33.6	33.9	130.2	242.1	164.6
No iron	106-5	12.0	12.4	10.3	39.1	39.1	30.5	275.0	282.6	137.3
injection	111-6	12.0	12.1	11.6	39.6	36.8	35.7	139.0	167.6	174.5
0 I.U. vit. E	106-10	9.3	11.2	11.0	35.7	33.2	32.0	187.8	3853.2	216.8
1000 mg Fe	106-4	10.1	8.9	11.6	38.2	29.0	36.8	187.8	2876.4	177.8
injection	Y1-3	9.2	8.7	11.7	34.4	33.1	35.1	239.3	3680.8	254.5
	111-7	9.5	9.9	11.2	36.1	36.7	38.6	205.0	3502.1	163.1
10 I.U. vit. E	121-8	11.2	9.6	11.1	32.5	34.5	34.8	247.0	241.8	195.8
No iron	106-2	9.8	10.0	10.7	36.3	19.8	33.1	184.0	219.3	241.9
injection	121-7	9.6	9.7	10.9	37.8	35.8	36.4	162.8	136.1	174.5
	108-7	12.1	10.3	11.9	37.2	30.0	36.4	175.6	223.6	214.0
10 I.U. vit. E	106-8	11.9	9.5	11.0	36.5	35.0	33.8	108.8	3051.9	175.5
1000 mg Fe	102-6	9.4	10.6	9.9	32.7	33.2	33.0	164.9	3312.1	168.3
injection	106-9	12.7	10.3	10.0	39.9	37.5	32.5	239.5	4663.6	200.0
	111-1	9.0	10.0	11.1	35.2	30.0	20.0	89.6	4652.6	208.4