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ABSTRACT

THE EFFECT OF FORM OF DIET ON IRON UTILIZATION BY THE BABY PIG

By

Roland L. Bjorklund

A trial involving 32 baby pigs from four litters was conducted to investigate the extent to which form of diet (liquid vs. dry meal) influences iron utilization by the baby pig and hence his iron requirement and to determine the comparative response to parenteral iron.

The pigs were weaned at an average age of 6 days and assigned (balancing for litter and sex) in groups of four pigs to either a semi-purified dry or liquid diet.

Within each form of diet there were groups of four pigs each receiving 50, 100 or 150 ppm of dietary iron from iron sulfate and a group that was given 100 mg of iron per pig from iron dextran injected intramuscularly at the start of the trial. Pigs on the liquid diet were fed 3 or 4 times daily. Pigs on the dry diet were fed ad libitum. The length of the trial was 3 weeks.

There was a significant effect of both form of diet and iron treatment upon the parameters of average daily gain,

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feed consumption, efficiency of gain, blood hemoglobin and hematocrit levels and serum iron level. Pigs on dry diets gained significantly faster but less efficiently than those on liquid diets. There was a positive relation of dietary iron level to pig performance. Pigs on liquid diets had significantly higher values of hematological parameters than those on dry diets. Only those pigs receiving 150 ppm of iron in liquid diets had values of hematological parameters equal to intramuscularly injected control pigs. There were significant form of diet x iron treatment interreactions for the parameters of growth, feed consumption and the efficiency of feed utilization. In all cases this interaction was due to the influence of iron treatment on the dry diet and there was no influence of iron treatment on these parameters on the liquid diet.

THE EFFECT OF FORM OF DIET ON IRON UTILIZATION BY THE BABY PIG

By

Roland LaFollette Bjorklund

A THESIS

Submitted to
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in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Animal Husbandry

1973

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The writer wishes expecially to acknowledge his deep gratitude to his wife, Natalie, whose encouragement and sacrifice made this study possible.

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The Effect of Form of Diet on Iron Utilization by the Baby Pig

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I. INTRODUCTION

A leading cause of mortality among baby pigs has been found to be due directly or indirectly to an insufficient amount of iron in the body of the young pig. Many aspects of swine production have undergone a striking change in recent years and, in some phases, phenomenal progress. The trend toward confinement production in many areas of the United States with the increased automated facilities and health protecting practices that have accompanied this type of production is well known. Progress in production goals has resulted in marked improvement in the rate of gain and in feed efficiency. It seems, therefore, that more attention and effort should be directed toward improving management practices and nutritional regimes that may have retarded progress in livability and improved growth rate in the young pig.

Since there are many nutritional factors which may influence iron absorption and utilization a review of these factors was made. Poorly known are the influences of physical factors in the diet upon nutrient absorption and utilization. Young pigs may be fed either liquid or dry diets and the research conducted in this study seeks to determine the influence of form of diet (liquid vs. dry meal) upon iron utilization by the baby pig.

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II. REVIEW OF LITERATURE

Comparisons of the digestibility of various products and certain nutrients in dry and liquid diets of young pigs have been investigated by different workers.

Protein and Protein Sources

The digestibility of soybean meal and dried skim milk in a dry diet by young pigs was conducted by Hays et al. (1959). Ration treatments included a basal soybean meal type diet and the basal plus D,L-methionine, basal plus L-arginine and basal plus D,L-methionine and L-arginine. Two milk type diets were fed which included the basal and basal plus L-arginine which equalized the arginine content to that of the soybean meal basal diet. Digestion of dry matter by the pigs on the milk diets at two weeks of age was significantly higher (96 percent) than the pigs on soya diets (88 percent). At five weeks of age the dry matter digestibility was 92 and 97 percent for the soya and milk diets, respectively. Much the same pattern existed in protein digestibility. The digestibility coefficients for soya protein was 78 and 82 percent for the two and five week age periods, respectively. Digestibility of milk protein remained

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constant at 96 percent. The effect of age on digestibility of both the dry matter and protein of the soya diet was statistically significant. The percentage nitrogen retention also showed interesting results with a considerably higher value (76 versus 51 percent) for the milk diets. At five weeks of age the figures became 58 and 50 percent with a significant change in the case of protein. The addition of arginine and methionine to the soya diets appeared to increase nitrogen retention at five weeks of age, but not significantly. Thus, the improvement demonstrated in the ability of the young pig during the period cited to digest dry matter and protein of a soybean meal ration would seem to reveal important changes in the gastrointestinal tract--probably enzymatic.

The digestibility of liquid, 28 percent protein, simulated milk diets by baby pigs was studied by Maner et al. (1959). The effects of pepsin and trypsin supplementation to soya protein diets on digestibility and weight gains were included. A comparison of casein and soya protein sources as to growth and digestion performance was also made. Percent protein digestibility was reported as follows: soya 89.8, soya + pepsin 90.9, soya + trypsin 90.6, soya + pepsin + trypsin 90.3, soya + pepsin in gelatinous capsule 89.0 and casein 96.6. Thus, enzyme supplementation had no significant effect on protein digestibility. Likewise, weight gains were not significantly different. These results in comparison to

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the digestibility of the soya protein in the dry diet by Hays et al. (1959) indicate that the soya protein when fed in a liquid diet has a higher digestion coefficient (89.8 percent) than soya protein when fed in a dry diet (about 80.0 percent). Differences in experimental procedure of the two experiments include the age of the pigs, 14 and 35 days in the case of the dry diet and 0 to 22 days for the liquid diet. However, it would seem that components of the ration and possible differences in soybean quality may alter the results as well as genetic constitution of the animals.

Diaz et al. (1959) studied the performance of baby pigs fed a commercially-prepared liquid infant diet as compared with pigs fed dry meal pig rations. Their results showed that performance favored pigs on the liquid diet. Feed efficiency was 1.22 kg gain/kg dry matter and 1.66 kg gain/kg dry feed and weight at five weeks was 10.0 and 7.6 kilograms, respectively, for pigs on the liquid and dry diets. The liquid diet contained 14.0 percent solids.

Carbohydrates

Included in the work of Cunningham and Brisson (1957a) was the digestibility of carbohydrate by baby pigs that were fed a semi-fluid diet including fish meal protein, glucose (cerelose) and lard. A fluid diet was also fed in which soybean protein replaced fish meal protein. The average apparent digestibility of glucose in percent ranged from 97.2 to 99.3 in the control group in which no enzyme was added.

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Studies by the same workers (Cunningham and Brisson, 1957b) dealt with the utilization of maltose by newborn pigs fed a fluid diet. Their results were very similar to the previous work cited. They found the digestion of maltose to range from 95.5 to 99.4 in percent digestibility. Glucose digestibility ranged from 99.6 to 100.0 percent.

The digestibility of the carbohydrate fraction of meal diets by young pigs was reported by Lloyd et al. (1957). In a diet composed of cereals, molasses, dried skimmed milk, soybean meal, fish meal, yeast and supplements, these workers found the apparent carbohydrate digestibility to be 90 and 91 percent at 3 and 7 weeks of age, respectively. However, a year later Lloyd and Crampton (1958) in using the same ration found the carbohydrate digestibility to be 85 and 80 percent. When these workers substituted wheat and meat meal for dried skimmed milk in the ration the apparent digestibility obtained was 80 and 84 percent.

On the basis of these studies it appears, therefore, that the carbohydrate fraction of the diet has higher digestibility by the young pig when fed in the liquid or semi-fluid form than in the meal form.

Their work was followed by Cunningham (1959) who tested the capacity of newborn pigs to digest raw corn starch and several of its degradation products fed in a liquid diet. Glucose, maltose and a soluble starch preparation were digested more rapidly than raw starch. Supplemental pancreatic

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amylase did not improve the rate of digestion of raw starch introduced directly into the intestine.

Fat

In earlier work Cunningham and Brisson (1955) found the apparent digestibility of lard in liquid diets for baby pigs to rise from 83.8 to 90.3 percent in the trial between the second and fourth week and from 91.3 to 97.6 percent in another trial. Digestibility was not affected by level of lard within the range 11.5 and 34.6 percent of the dry matter.

More recently the same authors (1957a) used 21.5 percent of lard in the dry matter in a liquid synthetic diet for baby pigs. They found the digestibility of lard was 81 percent at 2 to 9 days of age and 96 percent at 5 to 8 weeks of age in the ration that included 28.6 percent soybean protein and 35.7 percent glucose (cerelose).

Utilizing (dry) diets in work with pigs 10 to 20 days old, Sheffey *et al.* (1951) reported the digestibility of lard to be from 41 to 49 percent. However, it was believed these might be underestimates because of experimental errors.

The digestibility of several nutrients fed in a dry ration to pigs weaned at two weeks of age was reported by Lloyd *et al.* (1957). The apparent digestibility was determined at three and at seven weeks of age. The percentage digestibility of fat, which was 20 percent of the meal mixture, was as follows for 3 weeks and 7 weeks, respectively:

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short chain fatty acids--86, 96; medium chain--70, 90; long chain--37, 78. Average percentage digestibility of other ration components for the 3 and 7 week periods were: dry matter--84.4, 85.9; calories--85.8, 88.6; crude protein--85.3, 88.5; ether extract--39.1, 55.1 and total carbohydrates--90.1, 91.1. It is noteworthy that seven week old pigs digested fats and other components better than three week old pigs and the differences with age became wider as the molecular weight of the fats increased.

Additional work on the digestion of fat was reported by Lloyd and Crampton (1957). Pigs, guinea pigs and pups weaned at 14, 3 and 10 days were used in the trials. The average apparent digestibility in percent by pigs of fat or oil was as follows: short chain fatty acid 94.4, medium chain 91.9 and long chain 78.2.

A comparison of the results of the work of Cunningham and Brisson (1955, 1957), Sheffey et al. (1951), Lloyd et al. (1957) and Lloyd and Crampton (1957) seems to give strong indication that fat is digested by young pigs to a greater extent when fed in liquid diets as compared with dry diets.

Fat--Effect on Fe Absorption

Wissler et al. (1954) worked with hamsters in determining the effects of polyoxyethylene sorbitan monolaurate (Tween 20), which contains fatty acids upon iron absorption. The three experiments were from 8 to 20 weeks in duration.

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The results showed that hamsters fed a fortified bread ration containing 5 percent polyoxyethylene sorbitan monolaurate (Tween 20) showed a consistent increase in gastrointestinal absorption of a test dose of radioactive iron ^{59}Fe. Increases in the isotope were noted especially in the cecum and large intestine, the blood, bone marrow and liver. Further evidence of increased iron absorption was indicated since less radioactive iron appeared in the excreta of the Tween-fed animals than in the control animals.

Iron Requirements

The fact that the pig at birth contains relatively small amounts of stored iron and needs early replenishment has been known for many years. Likewise, researchers several decades ago reported their findings which showed sow's milk is too low in iron content to meet the requirements of the baby pig. McGowan and Crichton (1923, 1924) and Elvehjem et al. (1927) were among those who reported on work in this area. According to the National Research Council (1968), newborn pigs are reported to contain an average of 47 mg of iron and to require approximately 7 mg of absorbed iron daily for normal growth. Sow's milk has been reported to contain an average of 1.5 ppm iron (Miller, 1966).

Oral requirements of a baby pig as shown in the National Research Council (1968) publication in mg per kg of diet for different diets are: fortified cow's milk diet--60, dried

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skim milk diet--80 and purified casein diet--125. It is suggested that rations contain a minimum of 80 mg of iron per kg of diet.

The alarming number of baby pig deaths prior to weaning on many swine farms in the country should be reason for a thorough study of management practices. Krider and Carroll (1970) and others indicate losses of from 20 to 30 percent of pigs born alive in numerous swine producing areas. With the natural supply of iron (sow's milk) critically low, more attention and improved practices need to be directed to this phase of swine production as one of many causes of baby pig mortality.

In determining the requirement of the baby pig for orally administered iron, Ullrey et al. (1960) removed pigs from the sow when one week old and placed them on a preliminary diet of synthetic milk, unsupplemented with iron, for 4 days to one week. During the first trial the solids of a basal synthetic milk (20 percent solids) diet containing 25 ppm of iron was supplemented with zero, 10 or 100 ppm of ferrous iron. During the second trial the milk solids were supplemented with zero, 50, 100 or 200 ppm of ferrous iron. Experimental diets were fed for 6 weeks in all trials. Observations which were made at weekly or biweekly intervals included weight gain, hemoglobin concentration, hematocrit, erythrocyte count, reticulocyte count, leucocyte count, total serum protein, serum protein electrophoretic pattern, serum iron concentration,

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unbound iron-binding capacity of serum, total iron-binding capacity of serum and percentage saturation of transferrin with iron. They concluded that under the conditions 125 ppm of oral iron appeared to be adequate.

In a 42 day experiment Pickett et al. (1960) worked with pigs weaned at 10 to 14 days of age in determining oral iron requirements. Hemoglobin, hematocrit and red blood cell (RBC) determinations were made weekly. They found levels of 60 ppm iron or more in a dried skim milk basal ration gave normal growth. Erythrocyte concentration was reduced at levels up to 60 ppm iron while hemoglobin and hematocrit were reduced significantly at iron levels up to 80 ppm.

The same year Matrone et al. (1960) reported on their studies with baby pigs fed fortified cow's milk. They found that the minimum iron requirement up to 60 days of age was approximately 60 ppm of the dry matter intake. Utilization of iron at the minimum requirement level was approximately 30 percent. Also it was reported that iron utilization was not increased when baby pigs were brought to an anemic condition before initiating iron feeding. Tissue iron needs were met before hemoglobin iron needs when the pigs were critically deficient in iron.

Iron Sources for Weanling Pigs

Ammerman et al. (1969) studied the effect of iron sources on growth and hemoglobin responses of weanling pigs. Ferrous sulfate was compared with three ferrous carbonate ores which differed in their solubility in 0.4 percent HCl. Diets with ferrous sulfate supported greater ($P<.05$) gains than did the basal or basal + low solubility ferrous carbonate. Medium and high solubility ferrous carbonate diets supported higher ($P<.05$) average daily gain than did the basal diet. Final hemoglobin values were higher ($P<.01$) for the pigs fed ferrous sulfate than for those fed the basal, low or medium solubility ferrous carbonate. The high solubility ferrous carbonate supplement promoted higher ($P<.01$) hemoglobin levels than the basal or low solubility carbonate supplement.

Harmon et al. (1968) reported that corn would provide approximately half the recommended iron requirement based on a survey of corn samples collected in Illinois which contained a mean of 31 ppm iron. In their work with iron sources ferrous carbonate and ferrous sulfate were compared with the control group in a trial utilizing 16 pigs. The age of weaning was 3 days which was followed by feeding of a basal diet of 30 ppm iron until 15 days of age when they were allotted to the above treatments for 20 days. Their results showed ferrous sulfate to be an effective hematinic when fed to

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provide a total of 70 ppm of iron in the diet while ferrous carbonate failed to maintain hemoglobin in young swine at that level. Weight gains were not significantly influenced by dietary treatment, however.

In previous work Harmon et al. (1967) reported that ferric ammonium citrate was as efficient an oral hematinic as ferrous sulfate when fed to baby pigs in which the total iron provided was 127 and 126 ppm, respectively. These conclusions were based on hemoglobin values and weight gains.

Iron Metabolism

Progress in research in iron metabolism in the animal body received an impetus several decades ago with the production of radioactive iron. Austoni and Greenburg (1940) reported on work involving the absorption, distribution and excretion of iron in normal and iron-deficient rats. They found that the normal animals retained about 30 percent of the administered iron while the anemic animals retained 50 percent. The technique enabled the scientists to measure accumulation of absorbed iron per gram of tissue. Greatest amounts were found in the bone marrow, blood, spleen, liver and heart.

Previously it has been demonstrated by Moore et al. (1944) that the oral administration of ferrous compounds, as compared with ferric salts of iron, resulted in somewhat more rapid absorption of iron from the gastrointestinal tract.

Bergeim and Kirch (1949) worked with human subjects on the intragastric reduction to the ferrous form of ferric iron present in, or added to, ingested foods. A number of common foods such as breads, meats and fruits gave reductions as high as 50 to 90 percent. They reported that ascorbic acid, proteins, and protein digestion products appeared to participate in the reduction of iron.

Working in Japan, Furugouri (1973) investigated aspects of the mobilization of iron stores during the nursing period and the effect of parenteral iron administration on iron accumulation. Pigs were killed at birth, 24 to 30 and 72 to 84 hr. after nursing. At 3 days of age one group was allocated to iron dextran with 100 mg iron injected intramuscularly. The other group received no injection. Non-heme iron concentration in the spleen at birth was about half of that in the liver. At birth and 1 day of age there was more ferritin iron in the liver but less in the spleen, than hemosiderin iron. From 3 days of age onward, non-heme iron in the liver was mobilized markedly and ferritin iron dropped to a lower minimum level than did hemosiderin iron. With iron supplement, non-heme iron concentration in both tissues was markedly increased at 10 days and decreased linearly thereafter. Hemosiderin iron increased more than ferritin iron.

Variations in Plasms Iron and Total Iron Binding Capacity

Previously Furugouri (1971) revealed interesting data on variation of plasma iron and total iron binding capacity in pigs. Animals ranging in weight from 70 to 90 kg were used in the trial which was conducted in July, August and September. The results were as follows expressed in the range in values and the mean, respectively: Plasma iron (PI) 34 to 213 µg/100 ml, 123 µg/100 ml; total iron binding capacity (TIBC) 374 to 635 µg/100 ml, 540 µg/100 ml; percent PI, which is shown as the percent of PI to TIBC, 8 to 43 percent, 22 percent. Diurnal variation showed PI highest at midnight and 3 a.m. TIBC did not vary significantly during a day.

Gresham et al. (1971) working with mice studied the ratio of heme and non-heme iron uptake into erythropoietic tissues. They reported that the maximum proportion of heme ^{59}Fe in the spleen and femoral bone marrow of the mice is about 30 to thirty-five percent.

The contribution of maternal rat iron stores to fetal iron in maternal iron deficiency and overload was reported by Murray and Stein (1971). One group of rats was fed a diet of low iron content consisting of evaporated milk with a supplement of copper sulfate to produce iron deficiency. Another group was fed a normal laboratory diet and given an intramuscular injection of iron as iron dextran to produce iron overload. The workers found fetal iron content was preserved

in maternal iron deficiency but the fetus obtained more iron from maternal absorption. However, more of the fetal iron than normal was present as heme iron. In maternal iron overload, the fetal iron content was not increased but the fetus obtained less iron from maternal absorption. Most of this fetal iron was present as non-heme iron. The workers reported that on the average, 72 percent of fetal iron originates in maternal iron stores of which 89 percent is present as fetal non-heme iron. The implication is that 28 percent of fetal iron on the average arises from maternal absorption.

Meanwhile, Leslie and Kaldor (1971) working in Australia reported on their study involving body iron and some of its subfractions, heme iron, non-heme iron, ferritin iron and ferritin. These components were measured in groups of newborn rats and of rats aged 4, 15 and 24 days. Iron was found to accumulate in the rats throughout suckling, with slightly more going into the heme than the non-heme fraction. Heme iron was maintained well per unit body-weight throughout the study but total body iron and non-heme iron were both significantly diminished at 15 days. Within the non-heme fraction, ferritin iron was severely diminished at both 15 and 24 days but the non-ferritin portion of non-heme iron was increased. Body iron concentration did not differ significantly between newborn, 4-day-old and 24-day-old rats. However, body iron concentration of 15-day-old animals was significantly lower. Therefore, the results implied that by the end of the second

week of life, milk as the sole dietary source of iron had become inadequate in maintaining the iron status of the animal at the level at birth.

Copper

About 45 years ago Hart et al. (1928) demonstrated that copper may be used as a supplement to iron for hemoglobin regeneration in anemic rats. This early work on the action of copper in iron metabolism showed that in the presence of copper, soluble inorganic iron salts can be used directly for hemoglobin formation. This significant demonstration has led to studies by a number of workers concerning factors affecting hemoglobin production in rats and other species rendered anemic by whole milk diets.

Four years later Elvehjem and Sherman (1932) reported on their study of the action of copper on the storage and utilization of iron in rats. They found that the addition of pure iron to the milk diet of anemic rats which had been well depleted in their reserve of iron had no effect on the hemoglobin content of the blood but increased the total iron content of the liver and spleen considerably. When the iron content was replaced by copper, the store of iron in the liver was used directly for building blood hemoglobin. Inorganic iron $FeCl_3$ was found to be much more readily assimilated and stored in the liver than organic iron (hematin).

It was found that copper does not affect the assimilation of iron but does function in the conversion of inorganic iron into hemoglobin.

Copper (Interrelation)

The need for adequate copper to prevent anemia has been reported by Cartwright and Wintrobe (1948) who studied the relationship of plasma copper and plasma iron in normal and in pyridoxine-deficient sheep. The need for adequate copper to prevent anemia has been reported by these and others.

Hart et al. (1930) found that sows confined to pens and fed a yellow corn-skim milk diet maintained a normal hemoglobin level. The addition of varying amounts of iron and copper to the ration did not increase the hemoglobin level. Pigs farrowed by these sows developed a severe anemia in three to four weeks. The feeding of considerable amounts of iron and copper to the sow did not delay the development of anemia. The addition of pure $FeCl_3$ (copper free) to cows milk fed to the anemic pigs cured the condition. It was believed adequate amounts of copper were present in the tissue or were obtained under physical conditions of the experiment.

Lahey et al. (1952) in their study on hematological manifestations of copper and iron deficiencies in swine made these comparisons. The animals deficient in copper and those deficient in iron developed the following abnormalities: microcytic hypochromic anemia (more severe in iron deficiency),

normoblastic hyperplasia of the bone marrow, hypoferremia and an increase in the iron-binding capacity of the plasma. In addition the animals deficient in copper developed leukopenia, neutropenia, hypocupremia and reduced erythrocyte copper and tissue copper. The control group and copper-deficient animals received 30 mg of iron per kg of body weight daily. The control group and iron deficient pigs received 0.5 mg of copper per kg of body weight daily. Animals deficient in both iron and copper developed all of the manifestations of the copper-deficient pigs and also more quickly and to a greater degree. The interaction of these two elements appears to have therefore been demonstrated in hematology.

The work of Lahey et al. (1952) was followed shortly by Wintrobe et al. (1953) who reported on the function and metabolism of copper. A significant aspect of this work was their suggestion that copper is involved in the mobilization of iron from the tissues.

Work on the role of copper in the absorption of iron in swine was reported by Gubler et al. (1952). Working with copper-deficient swine they found several abnormalities in the metabolism of iron. Absorption of iron from the gastrointestinal tract was impaired. Mobilization of iron from the tissues was incomplete. The animals were unable to utilize parenterally administered iron for hemoglobin synthesis even when this element was presented to the bone marrow in normal quantities.

Evans and Abraham (1973) in a recent study compared rats that were fed a low copper (<1 ppm) and a control group that was fed 35 ppm as $CuCO_3$. Both diets contained 135 ppm iron as Fe_2O_3. Copper deficiency resulted in microcytic and hypochromic anemia which agrees with the work of Lahey et al. (1952). Evans and Abraham (1973) found that iron accumulated progressively in the liver. Absorption of iron in copper-depleted animals was, therefore, not altered. A common belief is that the iron entering the blood stream from the intestine is mostly in the ferrous form (Moore et al., 1939). More recently, Gaber and Aislen (1970) in their studies using ultraviolet difference spectroscopy have confirmed that a strong complex of iron-transferrin is formed only when the iron is in the trivalent state. The copper protein, ceruloplasmin, has a biological role here according to Osaki et al. (1966). These workers report that ceruloplasmin because of its ferroxidase activity oxidizes the ferrous iron into ferric iron before it is incorporated into transferrin. It is postulated, therefore, by Evans and Abraham (1973) that in the absence of ceruloplasmin the oxidation into the ferric form of iron is hindered. This in turn retards the formation of the carrier protein apotransferrin and thereby slows delivery to the site of hemoglobin synthesis. The decrease in ceruloplasmin oxidase activity which was evidently induced by the copper-deficient diet resulted in an increase in liver iron

accumulation and subsequently less iron available for hemoglobin synthesis.

The influence of copper on the absorption of iron has also been reported by Chase et al. (1952). In their work with rats, less absorption of iron from the gastrointestinal tract was found in the rats deficient in copper as compared with rats supplied with copper. Dietary copper supplied at levels of 0.25 to 0.50 mg per rat per day favored radioiron (^{59}Fe) absorption by the rat. At greater levels, somewhat reduced absorption of iron resulted. The influence of copper on iron absorption appeared to be correlated with the level of copper in the tissue.

These findings are in agreement with those of Ullrey et al. (1960) as far as liver tissue is concerned. Working with pigs, diets containing 6, 16 and 106 ppm copper were evaluated. Liver copper and iron concentration increased as dietary copper intake increased. The percent saturation of transferrin, albumin/globulin ratio and serum copper and iron concentration increased as copper intake was increased from the 6 ppm level to 16 ppm. The 16 and 106 ppm levels were not significantly different, however.

Results of work by Cassidy and Eva (1958) with pigs in England also showed that as copper concentration in the diet was increased from 125 ppm to 250 ppm to 500 ppm, copper concentration in the liver also increased accordingly. The concentration in ppm (average of each group) was 39, 154 and 558

respectively. Their results differed with those of Ullrey et al. (1960) however in the concentration of iron in liver since a decrease in liver iron of 118 to 92 to 36 in ppm was found as dietary copper was increased as indicated above. Ullrey et al. (1960) used considerably lower concentrations of copper in the diet, however.

Ritchie et al. (1963) in a study of copper and zinc interrelationships in the diet of weanling pigs obtained results similar to Cassidy and Eva (1958). They reported that as the concentration of copper in the ration was increased, iron storage in the liver decreased. Amounts of copper in the diets expressed in ppm were 13.1 (basal diet), 125 and 250. When added to the basal + 100 ppm zinc the copper concentrations were 125 and 250 ppm. It is interesting that the high calcium diet employed in the rations (1.3 percent) did not generally alter the inverse relationship between iron and copper concentration in the liver as reported by Cassidy and Eva (1958). In the work of Ritchie et al. (1963) the highest liver iron concentrations were obtained in the ration consisting of basal + 100 ppm zinc.

The role of iron in the copper-zinc interrelationship in the rat was also studied by Kinnamon (1966). He reported that diets high in copper or zinc do not significantly influence gastrointestinal absorption, tissue distribution or excretion of a single dose of iron administered orally 96 hours previously. However, diets high in zinc were found to significantly

lower the retention of chronically administered dietary radioactive iron as well as to decrease liver iron stores. It was postulated that zinc toxicity in time caused greater iron excretion due to a breakdown of red blood cells and a deviation from the normal metabolic pathway.

Bunn and Matrone (1966) from North Carolina State University studied the effect of cadmium, copper and zinc in the diet of male mice and male rats on liver iron. Cadmium was found to lower liver iron while zinc had the opposite effect. The latter result is noteworthy since other workers, Cox and Harris (1960), Magee and Matrone (1960) and Magee and Spahr (1964) report the reverse effect, that is, zinc decreased liver iron. (It is noted that Cox and Harris (1960) used both male and female rats.) The fact that Magee and Spahr (1964) and others used considerably higher levels of zinc (4000 to 7500 ppm versus 200 to 400 ppm) led to the postulation that zinc, depending on the level in the diet, may interfere in one or more steps in the hemopoietic system. This contention was given some support by the fact that when zinc without copper was added to the diet, iron accumulated in the liver, because the copper concentration was inadequate for normal mobilization and utilization of liver iron. The possibility that zinc increases iron absorption at low dietary levels and decreases iron absorption at high dietary levels was also suggested.

Manganese-Iron Antagonism

The effect of high dietary manganese in hemoglobin formation in lambs was studied by Hartman et al. (1955). Lambs on a whole milk diet and fortified with iron were given dietary treatments of manganese. A level as low as 45 ppm of manganese brought about a decrease in the concentration of hemoglobin and serum iron. Higher levels of manganese, up to 5000 ppm, were associated with decreased concentrations of iron in the liver, spleen and kidney. In a second experiment, anemic lambs were fed a roughage diet supplemented with three levels of manganese, 0, 1000, and 2000 ppm. Hemoglobin regeneration was markedly retarded and serum iron depressed in lambs fed diets containing either 1000 ppm or 2000 ppm of manganese.

Working with both mature rabbits and baby pigs, Matrone et al. (1959) found that excessive amounts of manganese at a level of 2000 ppm in the diet depressed hemoglobin formation in both species. A supplement of either 1250 or 2000 ppm of manganese in the diet also depressed growth. Hemoglobin regeneration of anemic rabbits on the high level of manganese was faster than that of the anemic baby pigs. The workers concluded that this was due at least partly to the fact that the baby pigs needed iron for hemoglobin repletion as well as for hemoglobin increases associated with growth. The minimal level of manganese in the diet that interfered with hemoglobin formation was estimated to be between 50 and 125 ppm.

Calcium

Kletzien (1940) studied the effect of calcium supplementation on a whole wheat ration fed to rats following a milk diet which produced severe anemia. The addition of 1 and 3 percent of calcium carbonate to the basal diet resulted in lower tissue iron values. Additional studies indicated that form of calcium in the diet was important in its influence on dietary iron utilization.

The need for adequate calcium in the diet for sufficient iron absorption for humans was later demonstrated by Apte and Venkatachalam (1964) in their work in India. Using a typical Indian diet of cereal as a basis for amounts of calcium and iron supplied daily it was found that with 400 mg of calcium intake, 16.6 mg of dietary iron was found insufficient to meet daily requirements in the presence of 40 percent phytate in the cereal diet. At 1000 mg of calcium about 4 percent of the 16.6 mg iron was absorbed, barely enough to meet the needs. Iron absorption increased to about 7 to 27 percent when 1500 mg of calcium were provided with 15.5 mg of iron. Thus, the beneficial effect of calcium on iron absorption was demonstrated.

Apte and Venkatachalam (1962) working in India reported on iron absorption in human volunteers using a high phytate cereal diet. Their results showed that an intake of 11.7 mg iron per day is insufficient to meet the requirements if the cereal diet contained 40 percent phytate. At a level of

16.43 mg iron intake daily, less than 1 percent was absorbed which was barely enough to meet the needs. When iron intake was brought to 21.55 mg iron daily, retention was nearly 30 percent of intake, which was adequate. They concluded that on a cereal diet with 40 percent phytate, the safe level of iron would be between 17 mg and 21 mg per day.

Cowan et al. (1966) studied the effect of phytate on iron absorption in the rat. Levels of 10 or 20 ppm of iron in the experimental diets were attained by mixing the appropriate amount of ferrous sulfate with the basal diet. For the phytate diets, sodium phytate was added at the expense of K_2HPO_4 at one of 2 levels: 0.7 or 1.2 percent by weight which represented 45 to 75 percent of the total dietary phosphorus, respectively.

In the experiment, 8 anemic rats were assigned to each of 6 diets. Diet groups 1, 2 and 3 were fed the diet containing 10 ppm of iron as ferrous sulfate; the other 3 groups received 20 ppm. Diets 1 and 4 were phytate free and served as controls. In diets 2 and 5, 45 percent of the dietary phosphorus was replaced by phytate phosphorus. In diets 3 and 6 phytate represented 75 percent of the total dietary phosphorus.

In the groups receiving 20 ppm of iron, hemoglobin regeneration was more rapid than in the groups given 10 ppm. However, at either level of iron, dietary phytate had no effect on iron absorption as measured by total hemoglobin regeneration.

Phosphorus

Some claims have been made that allowing piglets free access to iron and providing high dietary levels would stimulate gains. This led researchers to determine the toxic dietary level. O'Donovan et al. (1963) in working with early-weaned pigs found that a level of 5000 ppm iron significantly reduced rate of gain, serum inorganic phosphorus and femur ash. Iron levels of 4000 ppm resulted in a significant decrease in rate of gain, a slight decrease in serum inorganic phosphorus but did not reduce femur ash. Pigs fed 0.6 percent phosphorus, principally as defluorinated rock phosphate, and 5000 ppm iron developed typical phosphorus deficiency symptoms. Rate of gain, serum inorganic phosphorus and femur ash values were significantly reduced. However, these results were not noted when supplemental phosphorus was supplied by monosodium phosphate. High iron levels were found to be more toxic at the 0.3 percent phosphorus level than at either the 0.6 or 1.2 percent phosphorus levels.

When 5000 ppm iron was added to diets containing different phosphorus sources very constant serum inorganic phosphorus values were found when the animals were fed either dicalcium phosphate (U.S.P.) or monosodium phosphate. Animals that were fed steamed bone meal or dehydrated dicalcium phosphate did not show significant reductions in serum inorganic phosphorus values. However, the feeding of either defluorinated rock phosphate, Curacao Island Phosphate or dicalcium phosphate

(commercial) resulted in significantly lower serum inorganic phosphorus values.

Succinic Acid and Iron Absorption

The effect of succinic acid on iron absorption has been reported by Boddy and Will (1967) from work in Scotland. Three tests were conducted. Iron absorption of 20 mg ferrous succinate was compared with the absorption of 15 mg succinic acid added to the same amount of ferrous succinate. In 4 subjects iron absorption was increased by the addition of succinic acid. The difference was not statistically significant. When the amounts were increased to 150 mg ferrous succinate and 110 mg succinic acid only 2 showed increased absorption from doses containing the added succinic acid. Intravenous administration of 150 mg of succinic acid was given to patients prior to giving 5 mg ferric iron given orally. The results showed a decrease in iron absorption, as compared with subjects who were given 5 mg ferric iron without succinic acid.

Amino Acids

The influence of nine amino acids on iron absorption was investigated by Kroe et al. (1963). Working with male albino rats, nine amino acids and phosphate buffer alone were tested. Iron in the form of $^{59}FeSO_4$ with a specific activity of 25.1 mc/mg was used. It was concluded that the amount of

circulating iron is a reflection of three factors: (1) the amount and rate of absorption, (2) the amount and rate of deposition in the tissues, and (3) the release of iron from the tissues into the circulation. The results showed the amino acids could be grouped into three categories on the basis of patterns of the curves obtained for the rate and amount of ^{59}Fe appearing in the serum. Those associated with high blood ^{59}Fe values were glutamine, glutamic acid and asparagine. Lower values were associated with methionine, ethionine, proline, serine and phenylalanine. Histidine was associated with a much different pattern since the amount and rate of appearance of blood ^{59}Fe were the lowest at the beginning of the experiment but highest at the end (60 minutes after introduction of ^{59}Fe into the duodenum). All of the amino acids also effected an increase in iron deposition in the liver.

Kroe et al. (1964) in their work on the influence of histidine on iron and cobalt absorption in male rats found that histidine affected significantly the absorption of iron in the intestine when the pH ranged from 1.3 to 2.0 and 5 to 6.5 at the end of the 60 minute experimental period. The rate of absorption was greater at the lower pH level than at the higher pH. On the other hand, histidine inhibited significantly the absorption of cobalt with greater inhibition at a lower pH.

Continuing their work on the relationship of amino acids and iron absorption Kroe et al. (1966) included the interrelationship of pH and amino acids. Male albino rats were tested in which iron absorption was measured with and without histidine and glutamine in four separate pH ranges, namely, 1.3 to 2.0, 2.0 to 3.5, 5.0 to 6.5 and 7.0 to 8.0. In all pH ranges blood ^{59}Fe activity was higher when the intestinal segments were perfused with ^{59}Fe and histidine or glutamine than when an amino acid was not added. There was no significant difference between the blood ^{59}Fe levels of the glutamine groups and the histidine groups in any of the pH ranges at any time period, nor between the glutamine and control groups in pH range 2.0 to 3.5. In all other instances the ^{59}Fe blood levels of the histidine and glutamine groups were significantly greater than corresponding control group levels ($P<0.05$).

Vitamin A

Since two or more nutrients such as vitamins and minerals may be deficient simultaneously, the effects on the health of the animal in such circumstances is important. Amine et al. (1970) used male weanling rats in their work to study the hematological response during deficiencies of iron and vitamin A or both deficiencies together. Iron deficiency resulted in hypochromic microcytic anemia while vitamin A deficiency produced hypochromic microcytic polycythemia. Animals deficient

in both iron and vitamin A developed normocytic hypochromic anemia. Growth retardation occurred in all deficient animals with the double deficiency (both iron and vitamin A lacking) resulting in a greater reduction than when either was deficient singly. An interesting observation was the fact that a deficiency of iron resulted in a progressive decrease in both hemoglobin and red blood cell concentration while a lack of vitamin A caused both values to increase. When both nutrients were deficient, both hemoglobin and hematocrit decreased. It was postulated that the polycythemia due to a lack of vitamin A was present in this case but was masked by the anemia of the simultaneous iron deficiency.

Effect of Pyridoxine Deficiency on the Absorption of Iron by the Rat

It seems appropriate to cite studies concerning the rather unusual and interesting relation of pyridoxine and iron in the animal body.

Cartwright et al. (1944) demonstrated that the high serum iron values and hemosiderosis of the liver and spleen, which are characteristic of pyridoxine deficiency in swine, do not appear when the experimental animals are fed a diet very low in iron content, even though all other manifestations of pyridoxine deficiency develop. Thus, it is believed that in pyridoxine-deficient swine, iron continues to be absorbed even though it cannot be utilized and body stores are replete with iron.

Later, in work with rats, Gubler et al. (1949) found that the absorption of iron is increased during pyridoxine deficiency and that both iron and copper were significantly increased in the pyridoxine-deficient groups.

Additional work on the pyridoxine requirement of the baby pig was reported by Miller et al. (1957). Pigs from 3 to 5 days of age were weaned and fed a synthetic milk diet in which pyridoxine was absent. Concentrations of 0, 0.5, 0.75, 1.0 and 2.0 mg of pyridoxine per kilograms of solids were used in the diets of 5 lots of pigs.

It was reported that blood from pigs in all lots receiving vitamin B_6 was significantly higher in hemoglobin level ($P=0.01$) during the 3rd, 4th and 5th weeks than blood from the pigs receiving no B_6. It was concluded that the pyridoxine requirement of the baby pig is greater than 0.75 mg and perhaps less than 1.0 mg per kilogram of solids based on blood hemoglobin, red blood cell and lymphocyte counts and xanthurenic acid excretion. This is in agreement with work by Wintrobe et al. (1943) who indicate that pyridoxine deficiency arrests hemoglobin synthesis and leads to the development of a severe anemia.

Vitamin C

The role of ascorbic acid in the reduction of iron from the ferric to the ferrous form was studied by Kirch et al. (1947). Using an artificial gastric digestion technique, it

was found that fresh vegetables and fruits reduced the iron as much as 77 to 98 percent which was considerably greater than other foods. This was thought to be due to the action of ascorbic acid. Two years later Bergeim and Kirch (1949) used human subjects to determine the extent of reduction in iron forms by using different forms. Based on the extent of reduction by foods such as breads, meats and fruits it was thought that ascorbic acid, protein and protein digestion products participate in the reduction of iron.

Greenberg and Rinehart (1955) gave additional support to the vital role of ascorbic acid in relation to serum iron and anemia. Their work was with monkeys who had developed chronic vitamin C deficiency, hypoferremia and anemia. The oral administration of iron alone had no influence on the hypoferremia and produced only a slight to moderate increase in red blood cells and hemoglobin. The administration of both iron and ascorbic acid brought about marked improvement in both hypoferremia and anemia.

Vitamin C and E

The interrelationship of ascorbic acid and vitamin E was reported by Greenberg *et al.* (1957) in their work with rats. Greater stimulation of hemoglobin regeneration in iron-deficient rats was observed when iron was administered with both vitamin E and ascorbic acid than with either vitamin alone.

The substitution of N,N'-diphenyl-p-phenylenediamine (DPPD) for vitamin E in iron absorption and metabolism was studied by Tucker et al. (1957) employing young, iron deficient, milk-fed rats. Their results demonstrated the usefulness of this antioxidant in iron metabolism. The average levels of hemoglobin regenerated and the average hematocrits were higher in the group of rats supplemented with iron plus ascorbic acid and the antioxidant than in groups receiving iron alone or iron plus either ascorbic acid or DPPD.

Citric and Ascorbic Acids

Additional work on the role of ascorbic acid as well as citric acid in iron absorption was conducted by Hoppings and Ruliffson (1966). Their findings confirmed those of earlier studies in that ascorbic acid enhanced total iron absorption and also promoted retention of a greater proportion of the absorbed iron within the gut tissue. The addition of GSH (glutathione) reversed the tendency of retention of iron. It was suggested that ascorbic acid may also play a role in chelation which is important since certain iron chelates are thought to be absorbed as such.

This contention was confirmed in a study by Helbock and Saltman (1967) who supported the belief that chelates participate in the regulation and control of iron metabolism. In their explanation of the regulation and control of iron transport across the intestinal mucosa, two features were

proposed: (1) involvement of low-molecular weight chelating agents which are able to solubilize the iron and maintain it in a form for passage through the membrane, and (2) the presence of a carrier for transporting iron chelates in a mucosal→serosal direction.

Vitamin E

Scott et al. (1955) used a vitamin E-deficient basal diet in working with chicks. It was observed that the chicks displayed a microcytic anemia and a low reticulocyte count which indicated that vitamin E may be concerned in erythropoiesis.

Vitamin B_{12}

The vitamin B_{12} requirement of the baby pig was studied by Neuman et al. (1950). Optimum growth rates and highest hemoglobin values (14.3 g/100 ml blood) were obtained when pigs received 51 mcg/kg dry matter in the diet.

Effect of Diet

Amine and Hegsted (1971) reported on several dietary factors and the effects on iron absorption in iron-depleted rats. Maximum iron retention was observed in diets without salt mix. This was significantly higher than the retention from a diet supplying 2 percent salt mix ($P<0.005$) and iron retention with 2 percent salt mix was significantly higher

than with 4 percent salt mix ($P<0.025$). Iron retention from the diet with 4 percent phosphate-free salt mix was slightly lower than in the diet with the complete salt mix and iron retention in both was significantly higher than that in the diet with 4 percent calcium-free salt mix ($P<0.01$ and $P<0.05$, respectively). In addition, when dietary carbohydrate was varied, the order of iron retention (high to low) was lactose, lactose and starch, sucrose, glucose and starch with significant differences between diets. Finally, iron absorption from biologically labeled corn was higher than from liver or meat.

Work in India by Bhattacharya et al. (1964) on the metabolic relationship between dietary protein and iron produced interesting results. Male albino rat diets containing 0 to 18 percent protein were utilized. Their results showed that iron absorption is greatly impaired in animals fed protein-free diet or diets containing 3 percent protein. This was demonstrated since the storage of ferritin fraction of liver and spleen was lowered. Altering protein intake did not affect the efficient maintenance of plasma iron and blood hemoglobin levels except when protein was completely withdrawn from the diet. It was found that 6 to 9 percent protein in the diet is necessary to meet the normal needs of animals for iron absorption. This observation differs from that of Kalvins et al. (1962) who found approximately 15 to 18 percent protein in the diet necessary to meet normal needs for

iron absorption. It appears, however, from the report of Bhattacharya *et al.* (1964) that the quality of protein may be a factor in determining the level of protein intake for optimum efficiency of iron absorption.

III. EXPERIMENTAL PROCEDURE

A trial was conducted to study the influence of form of diet on iron utilization of the baby pig. Thirty-two pigs (Yorkshire, Hampshire, and Crossbreds) were weaned at an average age of 7 days and were assigned to either a semi-purified dry or liquid diet. Eight lots of 4 pigs each were balanced for litter and sex and randomly assigned to one of four iron treatment groups within either a dry or liquid diet. Thus, a 2 x 4 factorial was employed. Pigs were taken from the sow at 4 to 10 days of age and placed in individual stainless steel rearing cages equipped with stainless steel feeders and water troughs. Room temperature was maintained at about 30° C. The pigs were weaned to either a dry semi-purified diet or a liquid diet (Table 1). No difficulty was encountered in adapting to the liquid diet. The pigs adapted readily to the dry feed after small amounts of feed were placed in the animal's mouth.

The iron treatment for the 4 groups of 4 pigs each in both the liquid and dry diets are shown in Table 2. Desired level of iron in the diets was achieved through appropriate additions of $FeSO_4 . 2H_2O$.

The pigs on the low iron, basal liquid and dry diets (analyzing 2 µg of iron per gram of dry matter) received an

Table 1. Composition of experimental diets.

	Percent	
	Dry	Liquid[1]
Casein[2]	30	30
Glucose monohydrate[3]	55	58
α-cellulose[4]	3	--
Lard	5	5
Mineral mixture[5]	6	6
Vitamin mixture[6]	1	1

[1]Liquid diet made up to 20% solids with added water and homogenized at 8800 kg/cm^2 pressure.

[2]High protein casein, General Biochemicals, Inc., Chagrin Falls, Ohio.

[3]Cerelose, Corn Products Co., Argo, Illinois.

[4]Solkafloc, Brown Co., Chicago.

[5]See appendix Table 1.

[6]See appendix Table 2.

Table 2. Design of experiment.

Iron Treatment	Form of Diet	
	Liquid	Dry
IM[1]	4[2]	4
50 ppm	4	4
100 ppm	4	4
150 ppm	4	4

[1]A single IM injection of 100 mg Fe from iron dextran.

[2]Four individually fed pigs per cell.

injection of 1 ml (100 mg Fe) of iron dextran in the left ham at the beginning of the trial (two IM groups). Pigs on the dry diets were fed ad libitum throughout the trial and had free access to water, which was changed twice daily. All feed was weighed daily and individual feed consumption was recorded. Pigs on all levels of treatment on the liquid diet received 30 g of diet 4 times daily. This was gradually increased to 60 g of diet 4 times daily by the 4th day. On day 8, daily feedings were reduced to 3 and the amount increased to 75 g per feeding which was gradually increased for some pigs to 90 g and for others 120 g per feeding. The amount was regulated according to the condition of the pigs including normalcy of feces. Pigs were individually weighed weekly, July 16, July 23, July 30, and August 6 for the 21 day trial. Four pigs died during the trial, one each from the dry diet containing 50, 100 and 150 ppm Fe and one from the liquid diet containing 150 ppm Fe.

Diarrhea developed in several pigs and doses of 35 mg of neomycin[1] was administered orally as needed. The condition was more severe in animals on the dry diet. The severity is illustrated in Table 3.

Blood was taken from the anterior vena cava at the start and end (21st day) of the experiment and from an ear

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[1]Neomix, Tuco Products Co., Division of the Upjohn Co., Kalamazoo, Michigan.

Table 3. Antibiotic treatment for diarrhea.

Treatment Fe (ppm)	Animals per treatment receiving dosage of 35 mg of neomycin[1]	Dosage Number times treated per animal
50 (dry diet)	3	2, 4, 6
100 (dry diet)	2	4, 3
150 (dry diet)	2	1, 4
100 (liquid diet)	1	2
150 (liquid diet)	1	4

[1]Neomix, Tuco Products Company, Division of the Upjohn Company, Kalamazoo, Michigan.

vein on the 7th and 14th day for determination of blood and serum constituents.

Hematological Parameters

Hemoglobin

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

Hematocrit

Hematocrit was determined by the micro method (McGovern et al., 1955). Blood samples were centrifuged for 5 minutes at 10,000 RPM in an International "Hemacrit" centrifuge.

Analytical Procedures

Feed

A wet ashing procedure was used. For the dry diets sample weights of 3 g, 2 g, 1 g and 1 g were used for the four diets, namely, basal, 50 ppm iron, 100 ppm iron and 150 ppm iron, respectively. For the liquid diets the weights used for the diets in the same order were 15 g, 10 g, 5 g, and 5 g. The samples were placed in a 250 ml Phillips beaker and 60 ml of concentrated HNO_3 were added. This digestion mixture was heated on a hot plate until nearly dry and then allowed to cool. Seven ml of concentrated perchloric acid were added and digestion continued again to near dryness.

After cooling, samples were diluted to constant weight with de-ionized distilled water. Standards and blanks were prepared in an identical manner.

Iron content was determined by atomic spectrophotometry, using a Jarrell-Ash model 82-516 spectrophotometer equipped with a Hetco consumption burner. Samples were aspirated into an air-hydrogen flame. An absorption wavelength of 2480·5 Å was used.

Serum Determination

Blood samples from the pigs were collected in acid-washed test tubes and allowed to coagulate. After removal of the clot samples were centrifuged at 550 g for 15 minutes. The serum was then transferred to acid-washed vials and stored at -20° C.

Serum Iron

The procedure used in determining serum iron consisted of mixing 0.5 ml of serum and 1.0 ml of 20 percent of trichloroacetic acid (TCA) in a test tube. Following mixing in a vortex mixer the samples were heated for 15 minutes at 90° C and then centrifuged for 10 minutes at 550 g. Serum iron concentration was determined by atomic absorption spectrophotometry again using a wavelength of 2480·5 Å.

Total Iron-binding Capacity

The procedure developed by Olson and Hamlin (1969) was used to determine total iron-binding capacity. Equal volumes (1.0 ml) of serum and a ferric chloride solution (5 ppm iron) were mixed in a vortex mixer and allowed to stand for 5 minutes. Following the addition of 100 mg of magnesium carbonate the samples were mixed four times during a 30 minute period, and centrifuged at 550 g for 10 minutes. Then 0.5 ml of the supernatant was transferred to another test tube to which was added 1.0 ml of 20 percent TCA and the tube heated at 90° C for 15 minutes. Following cooling and centrifugation, the iron content of the supernatent was determined by atomic absorption spectrophotometry.

IV. RESULTS AND DISCUSSION

The response in performance to iron treatment and form of diet is shown in Table 4. The effect of form of diet on average daily gain and average daily feed intake was statistically significant ($P<.001$) and approached significance ($P=0.055$) on feed efficiency. The effect of iron treatment and iron interaction on these parameters were both statistically significant ($P<.001$).

The greater daily gain (116 g) of the pigs given 100 mg iron dextran initially on the dry diet as compared with the daily gain of those on the 50 ppm diet (-1.1 g) was readily apparent during the trial. In the latter group one pig died following a persistent diarrhea condition which affected other pigs in the lot to a lesser degree. The need for more antibiotics in this group is indicated in Table 3. The corresponding low feed intake of this lot paralleled the low rate of gain. The increased appetite of the pigs given intramuscular (IM) iron injection was consistent throughout the trial. This group also had a significant advantage in feed efficiency and had no death loss. One pig died in each of the other groups on dry diets.

In contrast, the pigs on different iron treatments on the liquid diet showed considerable uniformity in performance.

Table 4. Summarized pig performance data.

Form of diet	Dry				Liquid				Significance (P Value)		
Iron Level	Inj. 100 mg	50 ppm	100 ppm	150 ppm	Inj. 100 mg	50 ppm	100 ppm	150 ppm	Form	Iron	Form X Iron
Number of pigs	4	3	3	3	4	4	4	3			
Initial wt. (g)	2160	2206	2226	2373	2170	2085	2115	2217	0.937	0.736	0.898
Final wt. (g)	4598	2183	3107	2973	2473	2403	2525	2517	0.026	0.001	0.016
Average daily gain (g)	116^{d}	-1.1^{a}	43^{b}	29^{b}	14^{ab}	15^{ab}	20^{ab}	14^{ab}	0.001	0.0005	0.0005
Average daily feed (g)	173^{d}	87^{b}	125^{c}	115^{c}	47^{a}	44^{a}	46^{a}	44^{a}	0.0005	0.0005	0.001
Gain/Food	$.67^{c}$	$-.01^{a}$	$.34^{b}$	$.25^{b}$	$.31^{b}$	$.34^{b}$	$.43^{b}$	$.32^{b}$	0.055	0.001	0.001

^{abcd} Mean values in same row with different superscripts differ significantly ($P<0.05$).

Uniformity also prevailed in regard to visible health conditions. One pig died (150 ppm iron level) in the groups of pigs on the liquid diet. The average feed efficiency of the four groups on the dry and liquid diet was 31.5 and 35.0 percent, respectively. Average gain per day (g) was 46.7 and 15.8 and average feed consumed per day (g) was 125 and 45.5 by pigs on the dry and liquid diets, respectively.

Hematological data are presented in Table 5. A positive linear effect on both hematocrit and hemoglobin levels resulted with increases in dietary iron. This was true in both liquid and dry rations. In the dry ration, hematocrit levels increased through the second week in the groups given I.M. injection and 150 ppm dietary iron while the level remained about constant between the first and second week in the group receiving 100 ppm iron and dropped during this period on the low iron group. A very similar relationship is seen in the data from the liquid diet. Changes in hemoglobin levels during the three week period followed the same pattern as in hematocrit with the exception that the hemoglobin level of pigs on the 50 ppm diet increased slightly during the second week. Hematocrit levels declined during the third week in all lots of the four different levels of injected and dietary iron. The same week to week relationship existed in the hemoglobin levels as in hematocrits of the 4 groups on the dry diet.

Table 5. Hematological data.

Form of Diet	Dry				Liquid				Significance (P Value)		
Iron Level	Inj. 100 mg	50 ppm	100 ppm	150 ppm	Inj. 100 mg	50 ppm	100 ppm	150 ppm	Form	Iron	Form X Iron
Hematocrit, percent											
Initial	21.4	20.10	20.9	20.8	20.5	25.2	19.6	24.3	0.434	0.781	0.624
1 week	35.9	27.0	28.6	30.0	43.8	32.6	33.2	36.0	0.002	0.002	0.914
2 weeks	38.5	24.1	28.4	31.1	45.6	32.1	35.3	40.0	0.005	0.005	0.979
Final	31.4	19.6	21.9	23.9	39.8	28.5	29.3	30.8	0.0005	0.0005	0.973
Hemoglobin, g/100 ml.											
Initial	5.7	5.0	6.2	6.4	5.8	7.2	5.7	7.2	0.259	0.564	0.461
1 week	10.8	7.7	8.3	8.9	12.7	9.2	9.7	11.0	0.012	0.005	0.975
2 weeks	11.2	6.9	8.1	9.2	14.2	9.6	10.8	12.3	0.0005	0.001	0.970
Final	10.3	5.8	7.7	8.1	13.6	8.4	9.4	11.7	0.001	0.001	0.815
Serum Fe mcg/100 ml serum											
Initial	167	141	134	184	158	172	164	143	0.544	0.671	0.066
Final	164	115	86	177	177	84	200*	189	0.084	0.006	0.014
TIBC mcg/100 ml serum											
Initial	402	402	262	279	170	124*	242	270	0.054	0.548	0.681
Final	207	213	183	213	254	177	199	218	0.467	0.328	0.681
Transferrin saturation											
Initial	52	52	74	101	93	156*	95	89	0.091	0.529	0.423
Final	83	53	48	79	75	47	105*	92	0.347	0.564	0.235

*Questionable values since they are unusual and result in percent saturation values of over 100.

Table 6 shows the mean hematocrit and hemoglobin levels for each time period for all iron treatments on liquid and dry feeding.

The anemic condition of the pigs on the 50 ppm iron (dry diet) is indicated by hemoglobin levels of less than 8 g/100 ml throughout the trial and at the end of the trial of the pigs on 100 ppm diet. The anemia of the pigs receiving 50 ppm dietary iron was expected based on the work of Matrone et al. (1960) who considered that a dietary level of 60 ppm was necessary to maintain a hemoglobin level of 8 g/100 ml. Matrone et al. (1960) also suggested this level when pigs were fed a fortified cow's milk diet. Pickett et al. (1960) found a slightly higher dietary level to be necessary (80 ppm or more) to maintain normal hemoglobin and hematocrit levels when they fed a dried skim milk, semi-purified diet. Ullrey et al. (1960) recommended a level of 125 ppm in their work in which a synthetic casein-type diet was fed.

It is of particular interest to note that all the pigs on liquid diets at all levels of supplemental dietary iron maintained hemoglobin levels above 8 g/ml throughout the trial, thus demonstrating the positive effect in the utilization of iron by pigs fed a liquid diet.

As shown in Table 5 the effect on hematocrit and hemoglobin levels of form of diet and the effect of iron treatment were both statistically significant ($P<.001$). Form X

Table 6. Mean hemoglobin and hematocrit levels.

Parameter	Hematocrit, percent		Hemoglobin, g/100 ml	
Form of diet	Dry	Liquid	Dry	Liquid
Initial	20.8	22.4	5.8	6.5
1 week	30.4	36.4	8.9	10.7
2 weeks	30.5	38.3	8.9	11.7
Final	24.2	32.1	8.0	10.8

iron interaction was statistically significant on serum iron levels. Final serum iron levels were higher ($P<.01$) in the group on the liquid diet.

Some of the analyses on serum iron and TIBC were completed during the summer of 1972 and some were completed a year later. Storage temperature was approximately -20° C. It is possible that this may have influenced the values obtained on some samples which were not as expected as indicated in Table 5.

A comparison of the efficiency of iron utilization for hemoglobin formation by pigs on liquid and dry diets is shown in Table 7. The pigs on a liquid diet were significantly more efficient than those on the dry diet when treatment of 50, 100 and 150 ppm iron in the diet were compared. Table 4 indicates that the effect of form of diet, iron treatment and form X iron interaction were all statistically significant on rate of gain. Pigs on iron treatments in dry diets of 100 mg injected, 100 ppm and 150 ppm showed considerably faster gains than did those on liquid diets. This agrees with the work of Diaz et al. (1959). Lower hemoglobin levels can be expected in faster growing, larger animals since body size and blood volume is a factor in the concentration of this blood constituent in the animal body. These physiological differences are accounted for as explained in Table 7.

Table 7. Comparative efficiency of iron utilization for hemoglobin formation by pigs on liquid and dry diets.

Treatment	Hemoglobin formed, g[1]	Iron in Hb formed, mg[2]	Iron consumed, mg[3]	Efficiency of iron utilization, percent[4]
IM 100 mg				
Liquid	16.8	58.8	100	58.8
Dry	28.0	98.0	100	98.0
50 ppm				
Liquid	3.12	10.9	46.2	23.3
Dry	1.13	4.0	91.3	4.4
100 ppm				
Liquid	9.35	32.7	96.6	33.9
Dry	8.10	28.4	262.5	10.8
150 ppm				
Liquid	10.79	37.8	138.6	27.3
Dry	5.59	19.6	362.2	5.4

[1]Final Hb (g/100 ml) x Final wt. (g) x Blood volume (.08 ml/g wt.) - Init. Hb (g/100 ml) x init. wt. (g) x .08 ml/g wt. Hemoglobin turnover is ignored in calculations.

[2]Hemoglobin contains .35 percent iron.

[3]Either by intramuscular injection or from diet.

[4]Iron in hemoglobin formed (mg) ÷ iron consumed (mg).

Better utilization of protein by pigs on a liquid diet have been reported by Maner et al. (1959). It appears that the physiology of the baby pig is better prepared for efficient utilization of certain nutrients including iron when fed in a liquid diet. Perhaps enzymatic digestion of protein is more complete in liquid diets thus making amino acids available for iron chelation and making iron transport action more effective. A comparison of reports of other workers indicate superior utilization of carbohydrate and fat when fed in a liquid form.

The iron requirement of the baby pig therefore appears to be dependent to a degree on the form of diet. Table 5 indicates that a hemoglobin level of 8.4 g/100 ml was maintained at 3 weeks on 50 ppm iron in the liquid diet while in the dry diet 150 ppm iron resulted in only 8.1 g/100 ml.

Finally, the general health of the pigs fed dietary iron in the liquid diet appeared to be maintained at a higher level than those on dietary iron in the dry diet. This may be attributed to the reserve of nutrients available for body processes other than growth.

A statistical comparison of values for hematocrit, hemoglobin and final serum iron is shown in Tables 8, 9 and 10. In the final (3 week) hematocrit values (Table 8) the percent hematocrit of the pigs injected with 100 mg iron and fed a liquid diet was significantly higher than all other values. The value for pigs on the dry diet with 100 mg injected iron

Table 8. Statistical comparison of hematocrit values.

	Hematocrit, percent			
	Initial	1 week	2 week	3 week
DRY				
Inj. 100 mg	21.4	35.9^{b}	38.5^{bcd}	31.4^{d}
50 ppm	20.10	27.0^{a}	24.1^{a}	19.6^{a}
100 ppm	20.9	28.6^{ab}	28.4^{ab}	21.9^{ab}
150 ppm	20.8	30.0^{ab}	31.1^{ab}	23.9^{abc}
LIQUID				
Inj. 100 mg	20.5	43.8^{c}	45.6^{d}	39.8^{e}
50 ppm	25.2	32.6^{ab}	32.1^{b}	28.5^{bcd}
100 ppm	19.6	33.2^{ab}	35.3^{bc}	29.3^{cd}
150 ppm	24.3	36.0^{b}	40.0^{cd}	30.8^{cd}

^{abcde} Mean values in the same column with different superscripts are significantly different ($P<0.05$).

Table 9. Statistical comparison of hemoglobin values.

	Hemoglobin, g/100 ml			
	Initial	1 week	2 week	3 week
DRY				
Inj. 100 mg	5.7	10.8^{bc}	11.2^{cd}	10.3^{bc}
50 ppm	5.0	7.7^{a}	6.9^{a}	5.8^{a}
100 ppm	6.2	8.3^{ab}	8.1^{ab}	7.7^{ab}
150 ppm	6.4	8.9^{ab}	9.2^{abc}	8.1^{ab}
LIQUID				
Inj. 100 mg.	5.8	12.7^{c}	14.2^{e}	13.6^{d}
50 ppm	7.2	9.2^{ab}	9.6^{abc}	8.4^{ab}
100 ppm	5.7	9.7^{ab}	10.8^{bcd}	9.4^{bc}
150 ppm	7.2	11.0^{bc}	12.3^{de}	11.7^{cd}

^{abcde} Mean values in the same column with different superscripts are significantly different ($P<0.05$).

Table 10. Statistical comparison of final serum iron values.

	Final serum iron, mcg/100 ml serum
DRY	
Inj. 100 mg.	164[bc]
50 ppm	115[ab]
100 ppm	86[a]
150 ppm	177[c]
LIQUID	
Inj. 100 mg.	177[c]
50 ppm	84[a]
100 ppm	200[c]
150 ppm	189[c]

was significantly higher than all others on the dry diet. Pigs on the 100 and 150 ppm iron in the liquid diets had significantly greater hematocrit values than pigs on either the 50 ppm or 100 ppm iron in the dry diet.

Similarly, in Table 9, the hemoglobin value for the pigs on the liquid diet with injected iron is shown to be significantly greater than all other values except that of the pigs on 150 ppm iron in liquid diet.

Data on serum iron in Table 10 indicates that pigs on the 150 ppm iron in the dry diet and those with injected iron, 100 ppm and 150 ppm iron in the liquid diet had significantly greater values than those on 50 ppm and 100 ppm iron in the dry diet and 50 ppm in the liquid diet.

In this study increases in dietary iron resulted in a positive linear effect on both hematocrit and hemoglobin levels. Pigs on the liquid diet had a greater average feed efficiency while the gain per day and average feed consumed was highest in the pigs on dry diets. Finally, the efficiency of utilization of dietary iron in the formation of hemoglobin was highest in the pigs on liquid diets. The iron requirement of the baby pig in maintaining adequate hemoglobin levels appears to be dependent, therefore, on the form of diet.

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APPENDIX

Table A-1. Mineral mixture used in experimental diets.

Mineral	Percent
KCl	10.0
KI	0.002
$CuSO_4$	0.1
$CoCO_3$	0.1
$MnSO_4 \cdot H_2O$	0.1
$ZnSO_4 \cdot H_2O$	0.4
$MgCO_3$	2.0
$NaHCO_3$	25.0
$CaHPO_4 \cdot 2H_2O$	36.0
$CaCO_3$	12.5
Cerelose	13.798
	100.000

Table A-2. Vitamin mixture used in experimental diets.

Vitamin	ppm in diet
Thiamine mononitrate	3
Riboflavin	6
Nicotinamide	40
Calcium pantothenate	30
Pyridoxine hydrochloride	2
Para-amino benzoic acid	13
Ascorbic acid	80
D,L-α-Tocopheryl acetate	10
Inositol	130
Choline chloride	1300
	ppb in diet
Pteroylglutamic acid	260
Biotin	50
Cyanocobalamin	100
2-Methyl-1,4-naphthoquinone	40
Vitamin A palmitate	1500
Vitamin D_2	12.5


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