THE EFFECT OF DIETARY PROTEIN SOURCE ON IRON UTILIZATION BY THE BABY PIG

Thesis for the Degree of M. S.
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STUART AUSTIN LUMB
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ABSTRACT

THE EFFECT OF DIETARY PROTEIN SOURCE ON IRON UTILIZATION BY THE BABY PIG

by

Stuart Austin Lumb

Two trials, involving a total of 48 baby pigs, were conducted to investigate the effects of isolated soy or high protein casein on iron utilization by the baby pig.

Baby pigs were taken from the sow at 2 to 7 days of age and weaned to a dry purified diet. Blood samples were taken initially and at the end of each trial for hematological and serological analysis.

Pigs were housed in stainless steel rearing cages and were fed ad libitum.

Pigs were allotted to one of six dietary treatments. Iron supplied as $FeSO_4.2H_2O$ was added at 0, 50 and 100 ppm to provide three levels of iron. Supplemental iron was added to the casein diets to equate the iron content with those of the soy diets.

Mineral balance studies were conducted during the final two weeks of each trial.

As judged by growth and balance data, hematocrit, hemoglobin, erythrocyte and serum iron levels, the iron in the isolated soy diets was more available than that in the high protein casein diets. Consequently, under the conditions of the experiment, the iron requirement of the baby pig fed isolated soy protein is less than that of pigs receiving casein as the protein source.

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By

Stuart Austin Lumb

A THESIS

Submitted to
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DEDICATION

To the furtherance of Anglo-American understanding and to the betterment of international relations between all nations of the world

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The Effect of Dietary Protein Source on Iron Utilization by the Baby Pig

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I. INTRODUCTION

In the swine industry today there is a trend toward early weaning of pigs so that the sow may be rebred soon after farrowing to maximize her reproductive potential.

Several commercial swine producers, both in the United States and Europe, are rearing pigs which have been weaned at one week of age or less. For this practice to be successful, an economical early weaning diet must be developed. Most dry early weaning diets include milk products as the protein source. Because milk products are costly, it would be desirable to use less expensive soybean protein.

Many of the nutrient requirements of the early weaned baby pig, especially the mineral requirements, have been determined using purified diets containing casein as the protein source. Several workers have reported that the availability of zinc is less in diets containing soybean meal than in diets containing milk protein (Morrison and Sarett, 1958; O'Dell and Savage, 1960; Oberleas et al., 1962; Edwards, 1965). In addition, Miller et al. (1965) stated that baby pigs fed soy diets retained less calcium, phosphorus and magnesium than pigs fed casein diets.

In view of these reports, a study was undertaken to determine the effect of dietary protein source on the availability and utilization of iron by the baby pig. This question also has additional significance in that if the iron requirement of the baby pig can be met from dietary sources, the necessity of using an injectable form of iron, plus the inherent costs involved, could be eliminated.

II. REVIEW OF LITERATURE

Dietary Factors Affecting Iron Absorption

Minerals

Dietary iron level

The effect of the dietary iron level on absorption is directly related to the iron status of the individual under consideration. Thus, anemic individuals will absorb a considerably higher percentage of iron than a normal individual. This has been shown to be the case with humans (Pirzio-Biroli *et al.*, 1958); dogs (Stewart, 1953); chickens (Featherston *et al.*, 1968) and rats (Pollack *et al.*, 1964; Greenberger and Ruppert, 1966; Pearson *et al.*, 1967).

Ullrey *et al.* (1960) stated that the oral iron requirement for the baby pig was 125 ppm. Pigs fed diets containing 25, 35 and 125 ppm of iron absorbed 92.2, 71.4 and 49.9% of the iron, respectively, indicating decreased absorption with an increasing level of dietary iron. Matrone *et al.* (1960) also estimated the iron requirement of the baby pig. These workers estimated the utilization of dietary iron for hemoglobin synthesis and found that the utilization values for pigs receiving 40 and 80 ppm of dietary iron were significantly lower ($P<0.05$) than those for pigs receiving 10 or 20 ppm.

Valence form of iron

With reference to this topic, species differences do exist and therefore generalizations can only be made with regard to within-species comparisons.

Lottrup (1934) compared the effect of oral ferrous and ferric salts on hemoglobin levels in anemic children. He found that the ferrous forms were much more effective in increasing hemoglobin values. McCance et al. (1943) and Moore et al. (1943) also found that ferrous forms were more readily absorbed than the ferric, as judged by serum iron levels. Moore et al. (1944) used radioactive ferrous and ferric salts and estimated absorption by the amount of iron appearing in the hemoglobin. They found that the bivalent form was absorbed 1½ to 15 times more readily than the ferric form. Recent research from Holland would appear to cast doubt on the validity of the assumption that differences in the utilization of iron are a direct reflection of differences in absorption. Wiltink et al. (1966) measured the absorption and utilization of orally ingested ferric and ferrous salts. Absorption was calculated by the use of balance trials. Utilization of ^{59}Fe was calculated from blood volume and erythrocyte radioactivity. The authors, using both ferrous and ferric salts, found a significant negative correlation between intestinal absorption and utilization. It was further shown that patients given ferrous chloride or ferric versenate absorbed 20 or 22% of the dose respectively, although utilization of the ferrous chloride (32%) was considerably greater than that of the ferric versenate (9%). From this it would appear that absorption of the two forms is approximately equal. The authors also stated that the ferrous form was utilized to a greater extent than the ferric form, although in general it has been assumed that utilization of the two forms is the same.

In dogs, Moore et al. (1944) observed that the utilization of radioactive ferrous and ferric iron for hemoglobin synthesis was about equal although some dogs absorbed more of the ferrous form. The authors suggested that dogs may reduce the ferric form more efficiently than

humans. However, Hahn et al. (1945) using three anemic dogs found that there was greater uptake of the ferrous than the ferric form.

With rats, several workers have shown that both ferrous or ferric forms are equally available for hemoglobin regeneration in anemic animals (Austioni and Greenberg, 1940; Street, 1943; Blumberg and Arnold, 1947). Recent work by Fritz (1969) indicated that several forms of ferrous iron were considerably more available for hemoglobin repletion in anemic rats and chickens than ferric forms.

Because of the efficacy of injectable iron compounds as hematinics for pigs, research involving comparisons of oral forms of iron has been rather limited. Pickett et al. (1961) studied the availability of iron in different compounds using baby pigs reared on a dried skim milk semi-purified ration. In the three trials carried out, pigs receiving ferrous sulfate had significantly higher hemoglobin levels ($P<0.01$) than pigs receiving either ferrous carbonate or ferric oxide. Pigs receiving ferrous carbonate had higher hemoglobin levels than pigs receiving ferric oxide, although this difference was not significant. Harmon et al. (1967), also working with baby pigs, investigated the efficacy of ferric ammonium citrate as an oral hematinic. Using a semi-purified diet the authors concluded that, as judged by hemoglobin values and weight gains, ferric ammonium citrate was as efficient an oral hematinic as ferrous sulfate.

From the literature reviewed, ferrous iron, in general, is better absorbed than the ferric form. However, this may in fact be just a consequence of the greater solubility of the ferrous form. In order to resolve this matter, it would be necessary to compare the absorption of ferrous and ferric salts which had identical solubilities at a pH of around 6.5 to 7.0.

Iron salts

Nakamura and Mitchell (1943) measured the rate of hemoglobin regeneration in anemic rats fed various forms of iron salts. They reported that the iron in ferric chloride was twice as effective as that in ferric phytate for hemoglobin regeneration. In a similar experiment, Fuhr and Steenbock (1943) stated that the availability of iron from ferric phytate was 19% less than that of ferric ammonium sulfate for hemoglobin regeneration.

Also working with rats, Freeman and Burrill (1945) compared the retention of iron from several different sources. The compounds showed the following order of effectiveness in relation to hemoglobin regeneration: ferric chloride>sodium ferric orthophosphate=ferric phosphate>reduced iron sodium iron pyrophosphate. Blumberg and Arnold (1947) also found that ferric chloride was superior to ferric orthophosphate in terms of hemoglobin regeneration.

Working with pigs, Harmon et al. (1968) noted that ferrous carbonate was considerably less soluble than ferrous sulfate. They showed that a ferrous sulfate-supplemented diet (64 ppm of iron) gave significantly higher hemoglobin levels than a diet containing ferrous carbonate (70 ppm of iron). More recently, Harmon et al. (1969) stated that a survey of trace mineral salts currently used in swine diets indicated that ferrous carbonate was most commonly used to provide supplemental iron. In view of this, several experiments were conducted to further determine the efficacy of ferrous carbonate and ferrous sulfate. Pigs were fed a purified diet containing casein as the protein source. Hemoglobin values of pigs receiving ferrous sulfate were significantly greater ($P<0.01$) than those of pigs receiving an equal level of iron

(80 ppm) supplied as ferrous carbonate. Furthermore, the hemoglobin levels of pigs receiving the ferrous carbonate were equal to those of the control pigs receiving 29 ppm of iron. These data indicate that ferrous carbonate did not support normal hemoglobin levels, confirming the group's previous observations (Harmon et al., 1968). It was also shown that ferrous carbonate added to diets in varying amounts to give levels of 18 to 147 ppm of iron was ineffective in increasing hemoglobin levels. In addition, Fritz (1969) recently reported a relative biological value of zero for ferrous carbonate fed to chickens.

Research from the University of Florida also indicated that ferrous sulfate was superior to ferrous carbonate as a source of iron for the young pig. A diet containing 90 ppm of iron supplied as ferrous sulfate gave significantly higher hemoglobin levels ($P<0.01$) than diets containing 103 or 105 ppm of iron supplied as ferrous carbonate (Ammerman et al., 1969). The same group also compared samples of ferrous carbonate which differed in their solubility in acid as iron sources for young pigs. When fed with a basal diet, low, medium and high solubility ferrous carbonate diets gave hemoglobin levels of 7.3, 7.5 and 8.7 g/100 ml of blood. This would be expected as most of iron absorption occurs in the upper duodenum where the pH is still quite acid.

Thus, the differing availability of iron for absorption from the various salts is in part a consequence of their differing solubilities in acid media.

Calcium

Anderson et al. (1940) studied the effect of calcium on hemoglobin regeneration in anemic rats. The authors noted that increasing the dietary calcium level resulted in reduced iron absorption. Kletzien

(1940) also found that increased levels of calcium resulted in decreased iron absorption. The addition of 1% of calcium to a basal diet fed to anemic rats reduced the iron content of all tissues except the spleen. The average liver, blood and carcass values were 57, 86 and 90% of the respective control values. Increasing the level of calcium supplementation to 3% of the diet resulted in average liver, blood and carcass values which were 28, 72 and 61% of the respective control values.

Richards and Greig (1952) reported finding pale livers and enlarged flabby hearts in weanling mice whose mothers' diets contained 1 to 2% of added calcium carbonate. The dams fed a high level of calcium carbonate in the diet were also anemic. Continuing his research with mice, Greig (1954) carried out experiments to find if the anemia was due to calcium carbonate per se or to some other deficiency, induced by calcium carbonate. Substances which were considered likely to affect hemoglobin synthesis (e.g., copper and pyridoxine) were fed in excessive amounts along with calcium carbonate. However, all additives failed to prevent anemia developing. Consequently, Greig concluded that calcium carbonate exerted its anemigenic action through some interference with the assimilation or utilization of iron.

The effect of bone meal on the utilization of iron by anemic rats was investigated by Chapman and Campbell (1957a). Bread diets, supplemented with three levels of bone meal providing 3170, 4040 and 7330 ppm of calcium and six levels of ferrous sulfate (21.6 to 148.2 ppm of iron) were fed ad libitum to anemic rats over a 10-week period. With each level of iron fed, hemoglobin levels tended to be highest on the lowest level of bone meal and vice-versa. Furthermore, at all three

levels of bone meal fed, hemoglobin levels tended to increase with increasing levels of iron. Thus it would appear that increasing the amount of bone meal in the diet reduced the amount of iron available for absorption or utilization. In further work, Chapman and Campbell (1957b) carried out a study to determine whether the calcium or the phosphate portion of the bone meal was responsible for the interference with iron utilization. It was shown that there was no significant difference in hemoglobin regeneration of anemic rats fed a control diet (80% bread) containing 1670 ppm of phosphorus or of anemic rats fed the same diet supplemented either with disodium phosphate or sodium hexaphosphate, the phosphorus content of these two diets being 3620 and 3610 ppm respectively. However, the hemoglobin regeneration of anemic rats fed the control diet supplemented with either calcium carbonate (7900 ppm total calcium), or calcium chloride (7290 ppm total calcium) , was considerably less than that of anemic rats fed the control diet containing 3200 ppm calcium. Thus, under the conditions of the study, it was found that phosphate did not interfere with iron absorption, whereas calcium did.

Working in India, De and Basu (1949) found that human subjects kept on a diet of sago sugar and butterfat for 12 days almost maintained iron balance. However, when 500 to 1000 mg of calcium lactate was ingested with the diet, the subjects were in negative iron balance, mainly through an increase in fecal iron. It was concluded that the excess calcium was probably precipitated as insoluble calcium phosphate which removed a large amount of iron along with it. In further experiments with human subjects, Apte and

Venkatchalam (1964) compared the effects of three different levels of supplementary calcium, ranging from 360 to 1600 mg/day. The ratio of phytin phosphorus to total phosphorus was kept constant at 40%. With a daily intake of 400 mg of calcium, subjects on an iron intake of 16.6 mg were in negative iron balance. Subjects ingesting 1000 mg of calcium and 16.6 mg of iron/day absorbed 4% of the iron, while increasing the calcium level to 1500 mg/day resulted in 16% of the dietary iron being absorbed. It can be seen that increased calcium levels in this instance resulted in increased iron absorption. It is possible that at the higher levels of calcium supplementation, sufficient calcium was available to precipitate the phytate present in the diet. Consequently the iron, which might have been precipitated as iron phytate, was then available for absorption.

Very little research has been carried out related to the effects of calcium on iron absorption with swine. Working in Scotland, Greig (1960) fed young pigs a basal wheat and milk diet. Pigs receiving this diet had significantly higher hemoglobin levels at 8 weeks of age ($P<0.01$) than pigs receiving the same diet supplemented with 2% of calcium carbonate. Greig concluded that calcium carbonate reduced the availability of iron in the small intestine due to its antacid properties. However, there is a possibility that the reduction in absorption was due to the carbonate ion and not to the calcium. Pickett et al. (1961) showed that the availability of iron carbonate was much less than that of ferrous sulfate and its is possible that the reduction observed by Greig was due to iron carbonate formed in the intestinal tract.

In general, it can be stated that dietary calcium supplementation, regardless of species, reduces iron absorption, although a definite explanation for this occurrence appears to be lacking.

Phosphorus

Kinney et al. (1949) studied the influence of diet on iron absorption in the rat. The two basal diets, Purina dog chow and a corn grit meal, containing 350 and 27 ppm of iron respectively, were supplemented with an additional 3100 ppm of iron supplied as ferric citrate, giving four treatments in all. Liver iron determinations were made at the termination of the experiment, and it was found that the liver iron content of rats receiving the supplemented corn grit diet was considerably greater than that of rats receiving the corn diet alone (19.4 and 69.6 mg of iron/100 g of liver respectively). However, the liver iron content of rats fed the supplemented Purina diet was only slightly greater than that of rats receiving the unsupplemented Purina diet (9.0 and 13.9 mg of iron/100 g of liver). The authors noted that the liver iron content of the corn grit-fed rats exceeded the total body iron content of normal rats, and considered that the low phosphorus content (400 ppm) of the corn grit diet was responsible for this excessive deposition of iron. In further experiments, Hegsted, Finch and Kinney (1949) again working with rats fed a corn grit diet supplemented with 0.31% of iron, found that the amount of liver iron was inversely related to the phosphorus content of the diet.

Buttner and Muhler (1959) worked with Sprague Dawley rats which were fed a stock corn diet containing 0.32% of calcium and 0.31% of phosphorus. They found that a six-fold increase in liver iron

concentration occurred in rats receiving 50 mg of iron/day compared with the control group, which received no supplemental iron. However, the liver iron content of rats receiving a daily supplement of 50 mg of iron and 50 mg of phosphorus was significantly less ($P<0.0001$) than that of rats receiving supplemental iron alone (50 mg/day). Furthermore, the liver iron content of rats receiving 50 mg of iron and 100 mg of phosphorus/day was less than that of the group receiving 50 mg of iron and phosphorus/day.

O'Donovan et al. (1963) working with baby pigs, studied the effects of three different dietary phosphorus levels fed in conjunction with dietary iron levels of 80, 2500 and 5000 ppm. Levels of 1.2 or 0.6% phosphorus reduced the toxic effects of high iron levels as compared with 0.3% phosphorus supplementation. Consequently it would appear that in pigs receiving excessive amounts of iron and higher levels of phosphorus, much of the phosphorus was forming insoluble complexes with the iron, thereby rendering the iron unavailable for absorption and hence reducing the toxic effect.

Copper

It is well known that copper is essential for hemoglobin synthesis. Even with adequate supplies of dietary iron, if copper is lacking, anemia will develop (Cartwright et al., 1956). This indicates the involvement of copper in adequate iron utilization. Many workers have shown that copper deficiency anemia in swine can be successfully treated with copper supplementation, providing adequate iron is also supplied (Hart et al., 1930; Schultze et al., 1936; Lahey et al., 1952; Wintrobe et al., 1952).

Gubler et al. (1952) found that baby pigs fed a basal diet supplemented with 4.3 mg of iron and 0.5 mg of copper per kg of body weight per day absorbed 6.1% of an orally administered dose of ^{59}Fe, whereas pigs receiving the same level of iron, but no supplemental copper, absorbed only 1.85% of the labelled iron. As judged by plasma iron levels, it was also shown that little iron was absorbed in the absence of dietary copper, whereas in the presence of dietary copper, large amounts of iron were absorbed. Working in England, Cassidy and Eva (1958) fed bacon pigs diets containing 125, 250 or 500 ppm of copper. The liver iron values tended to decrease with increasing levels of copper in the diet, even though all pigs received the same level of dietary iron (400 ppm). Working with baby pigs, Ullrey et al. (1960b) compared diets containing 6, 16 and 106 ppm of copper. They found that 6 ppm of copper gave normal growth and erythropoiesis. Serum iron concentration was increased at the 16 ppm level, and liver iron content was shown to increase with increasing levels of dietary copper. In further work carried out at Michigan State, Ritchie et al. (1963) compared the effects of adding various levels and combinations of copper and zinc to a high calcium diet fed to growing pigs. The addition of 250 ppm of copper to the basal ration containing 13.1 ppm of copper and 42.2 ppm of zinc resulted in hemoglobin and hematocrit values which were significantly lower ($P<0.05$) than those from pigs on the basal ration. Workers at the Rowett Research Institute fed even higher levels of copper to pigs. Supplementation of a basal diet with 75 ppm of copper resulted in anemia developing, although pigs receiving the same level of copper with an additional 750 ppm of iron did not become anemic (Suttle and Mills, 1964).

Working with male albino rats, Chase et al. (1952) studied the effect of various levels of dietary copper (0.05 to 1.0 mg/day) on ^{59}Fe absorption and found that maximum iron absorption occurred with rats fed 0.25 to 0.5 mg of copper per day. It was also reported that the influence of copper on iron absorption was not due to the simultaneous administration of copper with iron, but appeared to be correlated with the copper level of the tissues. However, Kinnamon (1966) showed that there was no significant difference in liver iron content between rats fed a control diet containing no supplemental copper for 5 weeks and rats receiving the same diet supplemented with 0.02% copper over the same period of time.

Zinc

Magee and Matrone (1960) using rats, studied the effect of zinc on the absorption of ^{59}Fe. Results indicated that there was little difference in absorption between the control and zinc-fed rats, although the zinc content of the diets was not stated. Kinnamon (1966) also reported that there was no significant difference in the absorption of ^{59}Fe between rats receiving a diet containing 0.75% of zinc and rats receiving a diet containing no zinc supplementation. Also there was no significant difference in the amount of radioiron retained in the liver by the same two groups. Also working with rats, Bunn and Matrone (1966) found that dietary zinc levels of 200 and 400 ppm increased liver iron levels as compared to a basal level of 9 ppm of zinc. This finding is in contrast to the other reports quoted.

Regarding work with swine, Cox and Hale (1962) investigated the effect of 0.2 and 0.4% dietary zinc on liver iron in weanling pigs. The liver iron content of pigs receiving 0.4% of zinc was significantly

less ($P<0.01$) than that of the control group which received 40 ppm of added zinc. There was no significant difference in liver iron levels between the controls and pigs fed 0.2% zinc. The authors noted that even at the 0.4% zinc level, no toxicity problems were encountered, although growth rate was slightly reduced, as compared to the other two treatments.

Manganese

Hartman et al. (1955) stressed that as little as 45 ppm of supplemental manganese fed to anemic lambs resulted in decreased hemoglobin concentrations and serum iron levels. Higher levels of manganese, up to 5000 ppm, resulted in decreased iron levels in the spleen, liver and kidney. In a further trial, anemic lambs were fed a roughage diet supplemented with 0, 1000 or 2000 ppm of manganese. Hemoglobin regeneration was retarded and serum iron depressed in lambs fed diets containing either 1000 or 2000 ppm of manganese.

Further experiments to determine the effects of high levels of manganese on hemoglobin regeneration were carried out by Matrone et al. (1959), using rabbits and baby pigs. Supplemental manganese (2000 pm) depressed hemoglobin formation in both rabbits and baby pigs. However, a dietary supplement of 400 ppm of iron overcame this depressing effect. The minimum level of manganese in the diet that interfered with hemoglobin formation was 50 to 125 ppm.

Vitamins

Pyridoxine

Working with 30 lb pigs, Hughes and Squibb (1943) found that a diet deficient in pyridoxine resulted in poor appetite, reduced growth and the development of microcytic hypochromic anemia. On the addition of pyridoxine to the diet, however, the blood picture returned to normal. Miller et al. (1957) determined the pyridoxine requirement of the baby pig, using a synthetic milk diet. The hemoglobin levels of control pigs were significantly less ($P<0.01$) during the third, fourth and fifth weeks than those of pigs receiving pyridoxine. Pyridoxine is necessary for protoporphyrin synthesis and protoporphyrin is required for hemoglobin synthesis (Cartwright and Wintrobe, 1948). Consequently, the low hemoglobin levels observed by Hughes and Squibb and Miller et al. were due to a lack of protoporphyrin rather than to a lack of iron.

In contrast, Gubler et al. (1949) found that iron absorption of pyridoxine-deficient rats was greater than that of the control rats fed the same level of iron (1 mg/day). More recently, Neal and Pearson (1962) also working with rats, studied the relationship between pyridoxine deficiency and iron absorption at various levels of iron intake. In their first experiment, an iron-free diet was fed to control and pyridoxine-deficient groups for 5 weeks. All rats were given 0.1 mg of $^{55-59}Fe$ by stomach tube and killed 24 hours later. It was found that pyridoxine-deficient rats absorbed significantly less iron than the control group (P 0.05). In the next experiment, the two groups were fed iron-free diets for 10 weeks after which they were fed 0.1 mg of $^{55-59}Fe$/day for 14 days. In this case, there were no significant

differences in iron absorption. However, when $^{55-59}Fe$ was fed at a level of 1 mg/day over an 18-day period following 12 weeks on the iron-free diets, the pyridoxine-deficient group absorbed significantly more iron than the control group ($P<0.05$). The results of this last experiment are in agreement with those of Gubler et al. (1949).

In contrast, Shen et al. (1964) found that pyridoxine deficiency reduced iron utilization. Rats fed a pyridoxine-deficient diet containing 22.2 mg iron/100 g developed a severe microcytic hypochromic anemia after 40 to 50 weeks on trial. This anemia responded promptly to pyridoxine administration, hemoglobin levels recovering to normal after 2 weeks of pyridoxine therapy.

Vitamin B_{12}

Neuman et al. (1950) determined the vitamin B_{12} requirement of the baby pig fed a synthetic milk diet. Increasing levels of supplemental B_{12} gave increasing hemoglobin values, the highest value (14.3 g/100 ml blood) resulting from pigs receiving 51 mcg of B_{12}/kg dry matter. According to Wohl and Goodhart (1968) vitamin B_{12} is involved indirectly in deoxyribose nucleic acid formation. Consequently, if B_{12} is lacking the mitotic activity of the bone marrow is decreased, resulting in decreased erythropoiesis. In view of this, the increased hemoglobin values observed by Neuman et al. (1950) were probably a consequence of the effect of B_{12} on erythropoiesis.

Vitamin C

Moore (1955) showed that human subjects absorbed a greater percentage of ^{59}Fe when fed on a diet of eggs and citrus fruit juices than when fed eggs alone. The author suggested that ascorbic acid

present in the fruit juice increased absorption by promoting the reduction of ferric iron to the ferrous form. When ascorbic acid was substituted for the citrus juice, similar results were obtained. Other workers have also shown that foods containing appreciable amounts of ascorbic acid, such as tomatoes and orange juices, were effective in reducing ferric iron to the ferrous state (Kirch et al., 1947; Bergheim and Kirch, 1949).

The effect of ascorbic acid on iron absorption in rats was investigated by Groen et al. (1947). They also found that ascorbic acid increased iron absorption. It was concluded that the ascorbic acid effectively reduced intestinal pH and enabled more ferrous iron to be absorbed. Greenberg et al. (1957) further showed that the rate of hemoglobin regeneration was consistently greater in rats given supplements of iron and ascorbic acid than in rats supplied with iron alone. After observing that ascorbic acid resulted in increased iron absorption from ligated rat intestinal loops, Hopping and Ruliffson (1966) suggested that ascorbate-iron chelates may be formed and be responsible for the increased iron absorption. This was shown to be the case by Helbock and Saltman (1967). They showed that the uptake of ^{59}Fe by ligated rat intestinal segments was increased when an ascorbate-iron chelate was used. They stated that low molecular weight chelates are necessary to maintain iron in a soluble and permeable form.

Greenberg and Rhinehart (1955) observed hypoferremia and anemia in two rhesus monkeys exhibiting chronic vitamin C deficiency. Oral iron alone (50 mg/day for 41 days) had very little effect on this condition, but when supplemented with ascorbic acid the hypoferremia

and anemia were markedly reduced.

Vitamin E

Indovina (1951) found that rabbits exhibiting symptoms of vitamin E deficiency had low hemoglobin levels. The administration of 30 mg of vitamin E/day for 15 days resulted in increased hemoglobin values. Scott et al. (1955) developed a diet for use in the study of vitamin E deficiency in the chick. Birds receiving this basal diet displayed a microcytic anemia and a low reticulocyte count, and the authors indicated that vitamin E might be concerned in erythropoiesis. Working with primates, Day and Dinning (1956) found that anemia developed in rhesus monkeys fed a vitamin E-deficient diet. Injection of 20 mg of α-tocopherol phosphate resulted in the hematocrit value increasing from 30% to 44% over a 19-day period.

Greenberg et al. (1957) studied the effects of supplements of iron with ascorbic acid and vitamin E on hemoglobin regeneration in milk-fed anemic rats. They found that the rate of hemoglobin regeneration was consistently greater in rats receiving all three supplements than with iron alone or with iron plus either of the vitamins. In a continuation of this research, Tucker et al. (1957) substituted diphenyl-p-phenylenediamine (DPPD) for vitamin E in order to ascertain whether an antioxidant would stimulate hemoglobin regeneration. As in the previous work, milk-fed anemic rats were used. It was found that hemoglobin regeneration was greatest in the group receiving iron, ascorbic acid and the antioxidant as compared with groups receiving either iron alone or iron plus either ascorbic acid or DPPD.

Thus, it would appear that either vitamin E or a biologically active antioxidant, and ascorbic acid are interrelated and exert a considerable effect on iron metabolism in anemic rats.

Other Factors

Phytate

McCance et al. (1943) postulated that phytates interfere with iron absorption by precipitating iron as insoluble iron phytate, thus rendering the iron unavailable for absorption.

In balance studies carried out with human subjects, Widdowson and McCance (1942) found that more iron was absorbed from white bread than from brown, in spite of the fact that iron intakes were 50% higher on the brown bread diets. The reduced absorption from brown bread was attributed to its higher phytate content. Furthermore, the brown bread contained more inorganic phosphorus, and hence some iron could have been precipitated as ferric phosphate. McCance et al. (1943) working with human subjects noted that when large doses of ferric or ferrous ammonium sulfate were fed along with a standard breakfast of jam, and white bread containing sodium phytate, the increase in serum iron levels was less than when the iron salts and bread containing no added phytate were fed. Assuming that the rise in serum iron was proportional to the amount of iron absorbed, it was concluded that sodium phytate interfered with iron absorption by reacting with the iron as it passed through the intestinal tract and precipitating it as insoluble ferric phytate. Using seven different test meals, Sharpe et al. (1950) investigated the effect of phytate on the absorption of ^{55}Fe or ^{59}Fe. Using meals composed mainly of rolled

oats and milk, the authors stated that there was no correlation between the phytate content of the rolled oats and the reduction in iron absorption. Meals 4 and 7 were both composed of milk and rolled oats, the phytate content of meal 4 being approximately 50% higher than meal 7. The percentage of radioactive iron absorbed was 9.81 and 8.84 for the two meals respectively. It was pointed out, however, that these meals contained abundant amounts of calcium, quite in excess of the amount required to precipitate the phytate in the diet as calcium phytate. Thus, a possible explanation for the lack of correlation between the phytate content of the rolled oats and the reduction in iron absorption was the preferential combination of the calcium in the milk with the phytate, making it unavailable for combination with the iron in the meals. In the same experiment, when sodium phytate was added to a test meal, iron absorption was considerably reduced.

Indian workers have also shown that phytate exerts a considerable effect on iron absorption. Hussain and Patwardhan (1959) carried out several experiments with healthy male subjects. Cereal diets, with the proportion of phytate phosphorus to total phosphorus kept at 8% and 40%, were used. With an iron intake of around 22 mg/day, iron absorption was 11% and 3% for the two diets, respectively. Apte and Venkatachalam (1962), also working in India, found that an intake of 11.7 mg of iron/day was insufficient to meet requirements when a cereal diet, in which the proportion of phytin phosphorus to total phosphorus was kept at 40%, was fed to human subjects. Subjects on an intake of 16.4 mg of iron/day absorbed less than 1%; however, with an intake of 21.6 mg, retention was 30% and all subjects were in

positive iron balance. From this, the authors concluded that a satisfactory level of iron intake on cereal diets containing 40% phosphorus as phytate phosphorus appeared to be between 17 and 21 mg of iron/day. This level is nearly double that recommended by the United States Food and Nutrition Board (1968). From the above reports, it can be seen that phytate considerably reduces the amount of iron available for absorption. In contrast, Foy et al. (1959) using diets of bread, jam and sodium phytate fed to human subjects reported no consistent effect of dietary phytate on the absorption of radioactive ferric chloride. The authors considered that phytates per se had no adverse effect on iron absorption but that the products of their hydrolysis may include amounts of phosphates which would combine with the available iron in the intestine.

Apart from human subjects, rats have also been used to study the effect of phytate on iron absorption. Sathe and Krishnamurthy (1953) studied hemoglobin regeneration in anemic rats and concluded that phytin phosphorus inhibited iron absorption. However, neither the phytate nor the iron content of the diets used were reported, and the differences in values for hemoglobin regeneration amongst treatment groups were not statistically significant. In contrast, Harrison and Mellanby (1942) found that the addition of sodium phytate to the diet of anemic rats receiving 0.3 mg iron/day did not appear to inhibit hemoglobin regeneration when compared with the control group. More recently, Cowan et al. (1966) compared hemoglobin regeneration in groups of anemic rats fed purified diets containing 10 or 20 ppm of iron, in which either 45% or 75% of the total phosphorus was replaced with phytate phosphorus. Hemoglobin regeneration was more rapid in

the groups receiving 20 ppm of iron than in the groups receiving 10 ppm. However, the hemoglobin values showed that, even at the 10 ppm level, the rate of hemoglobin regeneration was not affected by the presence of either level of dietary phytate. In view of this, it was concluded that high levels of phytate have no effect on iron absorption in the rat.

Carbohydrates

Herndon et al. (1958) showed that D-sorbitol increased the absorption of ^{59}Fe in normal as well as anemic rats when compared with control animals. Rats received equal amounts of ^{59}Fe and D-sorbitol and it was observed that the amount of radioiron absorbed was a direct function of D-sorbitol concentration.

Fat

Wissler et al. (1954) studied the effect of polyoxyethylene sorbitan monolaurate (Tween 20), a fatty acid polyoxyethylene derivative of sorbitol, on iron absorption. Adult hamsters fed a fortified bread ration containg 5% Tween 20 absorbed greater amounts of radioiron than control animals. Increases were noted in the amount of isotope present in the cecum and large intestine, and results suggested that the excess iron was being absorbed largely in the cecum. The authors postulated that the increased iron absorption was due to absorption from the large intestine in addition to the usual absorption from the small intestine.

Organic acids

Groen et al. (1947) showed that the absorption of iron from closed loops of rat intestine was increased when solutions of citric, succinic and malic acid were administered. More recently, Boddy and Will (1967) studied the effect of succinic acid on the oral uptake of ^{59}Fe on human subjects. The absorption of a physiological dose of ^{59}Fe ferrous succinate was increased from 8.56% to 13.0% when succinic acid was given concomitantly with the ferrous succinate. According to Groen, the increase in iron absorption observed with the administration of organic acids was a consequence of the lowered pH. More iron would be in the ferrous form due to the lower pH and thus more iron would be in an absorbable form.

Protein and Iron Absorption

Dietary Protein Level and Iron Absorption

Klavins et al. (1959) showed that young male albino rats fed synthetic protein-free diets absorbed significantly less iron than pair-fed controls fed a diet containing 18% casein. Also the hemoglobin levels of rats fed the protein-free diet were lower than those of the controls. In conclusion, the authors suggested that a relationship existed between dietary protein level and iron absorption. More recently these same workers studied the effect of different dietary protein levels on iron absorption. Male albino rats were again used. It was found that approximately 15 to 18% protein was necessary for normal iron absorption. When lower levels of protein were fed, iron absorption was impaired, and the authors concluded that the dietary protein level exerted a definite quantitative effect on

iron absorption (Klavins et al., 1962). Bhattacharya et al. (1964), working in India, fed rats diets containing 0 to 18% protein. They also found that iron absorption and hemoglobin levels were greatly reduced in rats fed protein-free diets. The authors considered that a dietary protein level of 6 to 9% was necessary to maintain adequate iron absorption, which is considerably lower than that recommended by Klavins et al. (1962).

Effect of Protein Source on Mineral Absorption

O'Dell and Savage (1957) briefly reported that zinc in soy protein was less available than that in animal protein. Morrison and Sarett (1958) fed chicks diets containing either soy, or casein and gelatin, as the protein source. Adding zinc to the soy diet resulted in increased growth rate. However, adding zinc to the casein diet had no effect on growth rate. Removal of supplemental zinc from both diets resulted in reduced growth rates, although the effect was most marked in chicks receiving the soy diet. In view of this, the authors concluded that the soybean meal used in the experiment contained a factor which might have impaired the availability of zinc for absorption.

O'Dell and Savage (1960) studied the effect of phytic acid on zinc availability. Chicks fed a diet containing casein and gelatin as the protein source had much better growth rates than chicks fed a soy protein diet. However, when a casein-phytic acid complex and gelatin was used as the protein source, the growth rate was about the same as that on the basal soy diet. Both diets contained about the same amounts of phytic acid, phosphorus and zinc. When zinc was added to the casein-

phytic acid diet, growth rate was considerably improved. Chicks fed a soy protein-phytic acid complex exhibited zinc deficiency symptoms and grew even more slowly than chicks on the basal soy diet, although the addition of 15 ppm of zinc overcame the depressing effect of the added phytic acid. In conclusion, the authors suggested that the zinc in the isolated soy protein was less available than that in casein. Furthermore, they stated that from the results it appeared the phytic acid was involved in making zinc unavailable.

At the University of California, Davis et al. (1962a) carried out a study to determine whether soybean protein interfered with the utilization of various trace minerals. Chicks were fed diets containing three levels of zinc, copper and manganese, with and without ethylenediaminetetracetic acid (EDTA) along with isolated soy as the protein source. At each level of mineral supplementation, performance was superior in the EDTA-supplemented groups. The authors stated that EDTA is a strong chelating agent and that as such could form EDTA mineral complexes with the various trace minerals which were bound in the soy protein. These EDTA mineral complexes would then be available for normal absorption. No evidence was obtained that EDTA per se was growth stimulating.

More recently, Edwards (1966) studied the effect of protein source on the absorption of ^{65}Zn by the chick. It was found that chicks fed a casein-gelatin diet for 24 hours prior to dosing absorbed approximately 16% of an orally administered dose of ^{65}Zn. However, only 9% of the dose was absorbed by chicks on an isolated soy diet. ^{65}Zn was also given intraperitoneally. In this instance, there was only a slight difference in retention between chicks fed the different diets. Their

results would appear to indicate that soybean protein is able to reduce the availability of free zinc for absorption.

Several workers have also investigated the effect of soy and casein proteins on zinc availability and absorption in the pig. Smith et al. (1959) studied the effect of different protein sources on the zinc requirement of the growing pig. Pigs receiving isolated soy or soybean meal developed symptoms of parakeratosis, but no symptoms were observed in pigs receiving milk protein diets. This occurred even though the total dietary zinc content of the soy diets was higher than that of the milk protein diets. The addition of zinc to the soy diets resulted in significant growth increases ($P<0.01$) and alleviation of parakeratosis. The addition of zinc to the milk protein diets did not affect growth rate. Thus it would appear that for pigs, zinc in soy protein sources is bound and unavailable for absorption.

Oberleas et al. (1962) carried out experiments to determine the effect of phytic acid on zinc availability in growing swine. Over a 6-week period, pigs fed a basal soy protein diet containing 0.5% phytic acid gained significantly less ($P<0.05$) than pigs fed a basal casein diet. The zinc content of the two diets was 25 and 14 ppm, respectively. Pigs fed a casein diet supplemented with 0.7% phytic acid gained significantly less ($P<0.01$) than pigs fed the casein basal diet. However, when the casein-0.7% phytic acid diet was supplemented with 100 ppm of zinc, gains were superior to those on the casein-0.7% phytic acid diet and only slightly less than those achieved on the basal casein diet. Increasing the amount of phytic acid in the soy basal diet to 1.4% resulted in significantly reduced daily gains ($P<0.05$) as compared to those produced on the soy basal diet itself.

The growth depressing effect of added phytic acid was corrected by zinc supplementation, suggesting that the effect of phytic acid was to reduce the availability of zinc. This would explain the superior performance of pigs fed the basal casein diet over pigs fed the basal soy, even though the zinc content of the soy diet was nearly double that of the casein diet.

Working with the baby pig, Miller et al. (1965) compared casein and soy proteins with regard to calcium, phosphorus and magnesium balance. Balance data showed that pigs fed the soy diets excreted larger amounts of fecal phosphorus, calcium and magnesium than pigs fed the casein diets. The authors suggested that this difference was due to the poor availability of the phytate phosphorus in the soy protein and the formation of phytate complexes with calcium and magnesium rendering these cations less available for absorption.

Effect of Protein Source on Iron Absorption

Davis et al. (1962a) studied the effect of soybean protein on the utilization of trace minerals by the chick. As previously stated, the availability of zinc, manganese and copper was reduced in chicks fed the isolated soy protein diet. The availability of iron was also investigated in this same experiment. Diets containing 33.6, 43.6 and 58.6 ppm of iron were fed with and without a supplement of 0.07% EDTA. Addition of EDTA to the diets did not result in increased hemoglobin levels, indicating that EDTA did not increase the availability of the iron in the soy protein. If an EDTA-iron complex was in fact formed, it was not reflected in terms of changes in hemoglobin levels.

Further studies by Davis et al. (1962b) also showed that EDTA did not increase the availability of the iron in soy protein, thereby confirming the group's previous finding. They also stated that the iron in soy bean protein was found to be available for growth and hemoglobin formation. In addition, the availability of iron in soybean protein and dried skim milk was compared, the 2 basal diets containing 23.7 and 28.0 ppm of iron, respectively. Diets containing added iron (40, 80 and 160 ppm) were also fed. Hemoglobin levels of chicks receiving the dried skim milk diets were significantly higher ($P<0.0005$) than those of chicks on the soy diet. However, feed intake and consequently iron intake was greater on the dried skim milk diets, and the authors suggested that this was the reason for the higher hemoglobin levels of chicks receiving the dried skim milk diets. It was concluded that the iron in isolated soybean protein was approximately as available as the iron in dried skim milk.

More recent work on iron utilization and metabolism in the chick was carried out by the same group (Davis et al., 1968). The experiment was designed to determine whether the phytic acid contained in soybean protein interfered with iron absorption. Chicks were fed either an EDTA-washed soybean protein diet or a casein-gelatin diet, containing 24 and 25 ppm of iron, respectively for 14 days. Casein and soy diets supplemented with graded levels of iron (20 to 120 ppm) were also fed (see table 1).

In general, chicks fed the soybean protein diets had hemoglobin and hematocrit values equal to or higher than those of chicks receiving the casein-gelatin diet, and the authors concluded that: "no evidence was obtained that the soybean protein basal diet reduced the utilization of iron. Thus, the possibility that the phytic acid in the soybean protein interfered with the availability of iron was ruled out."

Table I. Effect of protein source on body weight and hemoglobin levels in chicks (14-day experimental period).

	Soybean diet		Casein-gelatin diet	
Dietary Iron ppm[a]	Body wt. g	Hb,g/100 ml blood	Body wt. g	Hb,g/100 ml blood
24	136.2	4.5	148.2	4.5
44	155.4	6.6	166.2	6.6
64	150.9	8.4	171.2	7.9
84	164.9	9.3	182.4	8.6
124	163.7	9.6	173.9	8.9
144	162.3	9.0	170.9	9.2

[a] The iron contents of the basal soy and basal casein-gelatin diet were 24 and 25 ppm respectively. Supplemental iron was added to give the higher iron levels.

Furthermore, EDTA-washed soybean protein was used in the trial. Previously, Davis et al. (1962b) showed that chicks fed an untreated soybean protein diet containing 45.3 ppm of iron had an average hemoglobin level of 6.5 g/100 ml of blood whereas chicks fed an EDTA-treated soybean diet (36.3 ppm of iron) supplemented with 10 ppm of iron had a hemoglobin level of 4.8. Thus it would appear that washing with EDTA in some way reduced the availability of the iron in the soy. In view of this, it could be assumed that had unwashed soybean protein been used in the 1968 soy/casein comparison, growth rates and hemoglobin values might have been even higher than those quoted (Davis et al., 1968).

Davis et al. (1962a) stated that isolated soybean protein contains a component which combines with zinc, manganese and copper and reduces the availability of these minerals. However, this was not the case with iron. In light of this statement it is interesting to note that Vohra et al. (1965) demonstrated that sodium phytate formed complexes with metals in the following decreasing order: Cu++, Zn++, Ni++, Co++, Mn++, Fe++ and Ca++.

Fritz (1969) stated that the iron in isolated soy protein was as available as that in ferric ammonium citrate, ferrous gluconate, ferrous fumarate and ferrous tartrate, and considerably more available than the iron in ferrous carbonate and various ferric salts. The repletion of hemoglobin and hematocrit levels in rats and chicks was used as the criterion to determine utilization of iron in the various sources tested.

In contrast to these previous reports, Fitch et al. (1964) found that 7 rhesus monkeys receiving purified diets containing isolated soybean protein became anemic after 2 to 7 months. Previously, monkeys fed casein diets containing the same amount of iron (210 ppm) had not become anemic.

Studies with radioiron showed that incorporation of ^{59}Fe from a soybean protein mixture was approximately 50% as great as that from a casein ^{59}Fe mixture. It should be noted however, that only one monkey was used in this study.

Thus, from literature reviewed, it would appear that in general, the iron in soybean protein is available for absorption and utilization.

Peptides and Iron Absorption

Mellander (1955) hypothesised that iron may combine with peptides and be absorbed in this form. He noted that peptides complex with iron and appear to act as effective chelating agents. An iron-peptide complex containing 20% iron given orally to humans was absorbed efficiently, as judged by increased serum iron levels. Indian workers showed that the ingestion of acid or enzyme hydrolysates of casein and iron increased the body iron in rats. They also suggested that peptides or other

protein degradation products acted as chelating agents and that peptides acted as vehicles for the transport of iron through the gastrointestinal mucosa (Bhattacharya and Esh, 1964).

Amino Acids and Iron Absorption

Rummel and Camdon (1956) stated that amino acids are good chelating agents and showed that an iron and alanine chelate, given orally, increased serum levels considerably more than ferrous sulfate alone.

Cysteine

Groen et al. (1947) noted that cysteine increased iron absorption from closed loops of rat small intestine. This effect could be due both to the reducing properties of the sulfhydryl group of cysteine and also to its chelating ability.

Methionine

Kaufman et al. (1966) fed methionine-deficient diets to rats for 4 weeks. Compared with controls, these rats developed anemia and their total body iron levels decreased, due to a decrease in iron absorption or retention. The low hemoglobin levels could also have been a direct consequence of the methionine deficiency, as hemoglobin itself contains a small percentage of methionine. This same group (Klavins et al., 1963) also studied the effect of excess methionine levels on body iron levels and hematological parameters. It was found that excess methionine feeding also produced anemia. To explain this result, the authors postulated that since methionine inhibits histidine absorption (Taylor et al., 1959) and, as histidine is the most limiting amino acid in hemoglobin synthesis (Borsook et al., 1957), the anemia observed was

a consequence of the unavailability of histidine due to the high level of methionine fed.

Other amino acids

Kroe et al. (1963a) showed that rats perfused with solutions containing ^{59}Fe and histidine absorbed more iron than rats perfused with the isotope alone. In comparisons involving other amino acids, however, histidine had relatively little effect on increasing iron absorption. Kroe et al. (1963b) compared the effects of various amino acids on iron absorption from the rat gastro intestinal tract, as measured by the appearance of ^{59}Fe in the serum and liver. Glutamine, glutamic acid and asparagine gave the greatest increases in blood ^{59}Fe values. Methionine, proline, serine and phenylalanine produced a moderate increase in iron absorption, and histidine supplementation increased iron absorption least of all. More recently the same group (Kroe et al., 1966) again showed that iron absorption was greater with glutamine supplementation than with histidine, regardless of pH.

In contrast, Van Campen and Gross (1969) found that histidine and lysine increased ^{59}Fe absorption from ligated rat duodenal segments, whereas glutamic acid, glutamine, methionine and glycine did not. In this instance, ferric iron was used, whereas in previous studies the ferrous form was prefered. This fact may partly explain the difference in results. Furthermore, the authors noted that their findings were consistent with the hypothesis that amino acid-iron chelates are formed and subsequently absorbed.

In conclusion, it is possible that the differences in iron absorption associated with different protein sources may in part be a consequence of their amino acid composition.

III. EXPERIMENTAL PROCEDURE

Introduction

Two trials were conducted to study the effect of protein source on the iron requirement of the baby pig. Two protein sources were used in conjunction with three levels of dietary iron; four pigs were allotted to each treatment group, thereby giving a 2x3 factorial.

General Conduct of Experiments

The two trials involved a total of 48 pigs, all from the University herd. In trial 1, 20 Yorkshire and four Hampshire pigs were used; in trial 2, pigs were all of the Yorkshire breed.

The experimental procedure was the same in both trials unless otherwise stated. Pigs were taken from the sow at 2 to 7 days of age and placed in individual stainless steel rearing cages equipped with stainless steel feeders and water troughs. Room temperature was maintained at 20° C for the duration of the trial. The pigs were weaned to a dry purified diet (table 2). Intake was encouraged by placing small amounts of feed in the animals' mouth. The pigs readily adapted to the dry feed and no problems with adaptation were encountered.

The pigs were fed a low iron diet (15 ppm) for several days to deplete their iron reserves after which four pigs were allotted to each treatment group on the basis of sex, weight and litter. The experimental diets contained either purified casein or isolated soy as the protein source. The soy protein diets were supplemented with 0.3% DL-methionine. A basal diet, basal plus 50 ppm of iron and basal

Table 2. Composition of experimental diets.

	Casein[1]	Soy[2]
	%	%
Casein	30	-
Soy	-	30
DL-Methionine	-	0.3
α-Cellulose[3]	5	5
Lard	5	5
Cerelose[4]	51	50.7
Mineral mixture[5]	6	6
Fat-soluble vitamins in corn oil[6]	1	1
Water-soluble vitamins in water[6]	2	2

[1]
[2] High Protein Casein, General Biochemicals, Chagrin Falls, Ohio.
[3] Soya Assay Protein, General Biochemicals, Chagrin Falls, Ohio.
[4] Solka Floc, Brown Company, Chicago, Illinois.
[5] Cerelose, Corn Products Company, Argo, Illinois.
[6] See Appendix Table 1.
See Appendix Table 2.

plus 100 ppm of iron were produced, using $FeSO_4 \cdot 2H_2O$ for supplementary iron. On completion of trial 1 it was found that the iron content of the soy protein was considerably greater than the assumed value. Consequently the iron content of the soy diets was greater than that of the casein diets. In view of this, in trial 2 the amount of supplemental iron in the casein diets was increased in order that the casein and soy diets would contain equal amounts of iron at the three treatment levels. The analyzed iron content of the diets is shown in table 3:

Table 3. Dietary iron levels (ppm).

	Basal		Basal+ 50 ppm Fe		Basal+100 ppm Fe	
	Casein	Soy	Casein	Soy	Casein	Soy
Trial 1	45	73	88	137	152	189
Trial 2	101	95	148	147	189	189

Blood was taken from the anterior vena cava initially and at the conclusion of each trial for determination of blood and serum constituents.

Pigs were fed ad libitum and had free access to water, which was changed twice daily. All feed was weighed out daily and individual feed consumption was recorded. Pigs were individually weighed weekly for the duration of the trial, following which they were returned to the University herd. Pigs were kept on trial 1 for 29 days and for 35 days in trial 2.

Mineral balance studies were conducted during the final 2 weeks of both trials. Two pigs from each treatment group were selected on the basis of equivalent weight and placed in individual metabolism cages. Pigs were removed from the cages three times daily and individually

fed an amount of food and water which could be consumed within a 5 to 10 minute period. Following this, the pigs' mouths were wiped clean to avoid contamination of excreta. The pigs were then returned to the cages. After a 3-day adjustment period, fecal and urine collections were made over a 3-day period. Feces were separated from urine by means of a fine screen placed above the collection funnel. Where possible, constant daily feed intakes were maintained throughout the balance period.

Feces were oven dried for 24 hours, weighed, ground and stored in sealed plastic containers. Urine was collected in polyethylene containers and acidified with 6N HCl. Following the collection period, the urine volume was recorded and 100 ml aliquots were taken and stored in acid-washed polyethylene bottles at 3° C.

During each trial, one pig on the basal casein diet went off feed, lost weight became weak and was consequently killed. Post mortem information was not obtained on pig 57-5 which died during the first trial. A post mortem examination of pig 17-7 which died during the second trial revealed evidence of encephalomalacia and meningitis.

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.

Erythrocytes

Erythrocytes were counted in duplicate from a single filling of a "zero error" Hellige pipette, using an improved Neubauer counting chamber. The diluent used was 0.85% NaCl.

Reticulocytes

Four drops of methylene blue were mixed with an equal amount of blood and allowed to stand for 10 minutes. Following this, duplicate thin smears were made, allowed to dry and then stored for later counting. Reticulocytes were enumerated per 1000 erythrocytes and expressed as a percentage.

Analytical Procedures

Feed

A wet ashing procedure was used. A 1 g sample was 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 to near dryness and 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 deionized distilled water. Standards and blanks were prepared in an identical manner.

Iron content was determined by atomic absorption spectrophotometry, using a Jarrell-Ash model 82-516 spectrophotometer equipped with a

Hetco total consumption burner. Samples were aspirated into an air-hydrogen flame. An absorption wavelength of 2480.5 Å was used.

Feces

Fecal iron was determined by an identical procedure, except that a sample weight of 0.5 g was used.

Urine

For iron determinations, undigested and undiluted samples were used and concentrations were determined using atomic absorption spectrophotometry.

Serum Determinations

Blood samples from the pigs were collected in acid-washed test tubes and allowed to coagulate. Following removal of the clot, samples were centrifuged at 550 g for 15 minutes. The serum was then transfered to acid-washed vials for 5° C storage.

Serum protein analysis

Total serum protein was determined using a modified Lowry procedure (Miller, 1959). Electrophoretic separation of serum proteins was achieved using agar strips in a modified Beckman-Spinco Durrum cell. Strips were scanned using a Beckman RB Analytrol densitometer.

Serum samples were then frozen and stored at -10° C until further analyses were performed.

Serum iron

Serum iron concentration was determined by atomic absorption spectrophotometry, again using a wavelength of 2480.5 Å. Serum

samples were diluted 1:1 with deionized distilled water prior to serum iron determination.

Total iron-binding capacity

The procedure developed by Olson and Hamlin (1969) was slightly modified and used to determine total iron-binding capacity. Equal volumes (0.5 ml) of serum and a ferric chloride solution (5 ppm iron) were mixed and allowed to stand for 5 minutes. Following the addition of 50 mg of magnesium carbonate, samples were mixed four times during a 30 minute period, centrifuged and 0.5 ml of the supernatant transferred to another test tube. One ml of 20% TCA was added to the supernatant, and the tube heated at 90° C for 15 minutes. Following cooling and centrifugation, the iron content of the supernatant was determined by atomic absorption spectrophotometry.

Hemoglobin iron

Because of hemolysis in certain serum samples, it was necessary to correct for the hemoglobin iron present in these samples. The spectrophotometric method for quantitating hemoglobin in serum developed by Hunter et al. (1950) was used.

Statistical Analysis

The data were analysed by analysis of variance and by the multiple range test of Duncan (Bliss, 1967).

IV. RESULTS AND DISCUSSION

Trial I

Pig performance data are presented in table 4. In general, a positive linear effect was noted with an increase in the iron content of the diets, irrespective of the protein source. Differences in average daily food intake approached significance ($P<0.07$) with regard to dietary iron level.

Hematological data are presented in table 5. Regardless of protein source, hematocrit values increased with increasing dietary iron level ($P<0,001$). The effect of protein source on hematocrit values approached significance ($P<0.07$). Hemoglobin levels similarly significantly ($P<0.001$) increased with increasing dietary iron level. The effect of protein source on hemoglobin levels was not significant. As judged by hemoglobin levels, pigs on the 45 ppm casein and 73 ppm soy diets were anemic, having hemoglobin levels of less than 8 g/100 ml. This is not unexpected as the Agricultural Research Council of Great Britain (1967) considered that a dietary level of 60 ppm was necessary to produce a hemoglobin level of 8 g/100 ml. This is the same level as suggested by Matrone et al. (1960) for baby pigs fed a fortified cows' milk diet.

Pickett et al. (1960) using a dried skim milk semi-purified diet found that normal hemoglobin and hematocrit levels were obtained with a dietary level of 80 ppm or more. Ullrey et al. (1960a)recommended a level of 125 ppm for pigs fed a synthetic casein-type diet. Consequently, the type of diet fed influences the oral iron requirement.

Table 4. Summarized pig performance data, trial 1.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	45	88	152	73	137	189	±SE[1]	Prot.	Iron	Repl.	Prot.xiron
Initial wt., kg	2.1	2.0	2.1	2.1	2.1	2.1	0.19	0.93	0.92	0.30	0.94
Final wt., kg	4.7	7.9	7.4	6.8	7.5	9.4	1.34	0.27	0.16	0.48	0.56
Avg daily gain, kg	0.09	0.20	0.18	0.16	0.19	0.25	0.04	0.24	0.13	0.54	0.50
Avg daily feed, kg	0.17	0.24	0.25	0.22	0.23	0.32	0.03	0.20	0.07	0.19	0.51
Gain/food	0.52	0.83	0.68	0.69	0.77	0.80	0.11	0.41	0.24	0.68	0.56

[1] Standard error of the mean.

Table 5. Hematological data, trial 1.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	45	88	152	73	137	189	±SE[1]	Prot.	Iron	Repl.	Prot.xIron
Hematocrit, %											
Initial	26.9	28.7	25.0	31.3	27.0	26.9	1.86	0.33	0.26	0.11	0.30
Final	20.8	29.0[aa]	33.0[aa,b]	26.3[aa]	29.3[aa]	34.4[bb,d]	1.53	0.07	0.00	0.89	0.24
Hemoglobin, g/100 ml blood											
Initial	7.3	8.3	6.7	8.7	7.8	7.5	0.60	0.32	0.23	0.23	0.30
Final	5.4	8.4[a]	9.1[aa]	7.4	8.3[a]	9.6[aa]	0.69	0.18	0.00	0.43	0.32
Mean corpuscular hemoglobin concentration, %											
Initial	27.1	29.2	26.7	27.7	28.8	27.8	0.76	0.50	0.07	0.21	0.62
Final	26.1	28.8	27.6	28.3	28.1	27.7	1.27	0.63	0.62	0.20	0.51
Erythrocytes, $10^6/mm^3$											
Initial	4.01	4.67	3.72	4.72	3.74	4.44	0.50	0.50	0.66	0.06	0.14
Final	6.08	7.30	6.93	6.73	6.26	6.22	0.45	0.33	0.72	0.81	0.17
Mean corpuscular volume, cubic microns											
Initial	71.9	61.3	78.1	74.0	79.6	61.1	11.86	0.91	0.96	0.46	0.35
Final	34.1	40.2	48.3[a]	39.2	47.1[a]	56.7[cc]	3.52	0.03	0.00	0.92	0.90
Reticulocytes, % of erythrocytes											
Initial	2.5	2.7	3.1	2.2	3.4	3.6	0.58	0.54	0.25	0.04	0.60
Final	2.0	1.4	2.4	1.7	1.3	1.4	0.45	0.28	0.44	0.26	0.61

[1] Standard error of the mean.
[a] Significantly greater than least value ($P<0.05$); [aa] ($P<0.01$).
[b] Significantly greater than least two values ($P<0.05$); [bb] ($P<0.01$).
[c] Significantly greater than least three values ($P<0.05$); [cc] ($P<0.01$).
[d] Significantly greater than least four values ($P<0.05$); [dd] ($P<0.01$).

Differences in mean corpuscular hemoglobin concentration and erythrocyte counts between treatment means were not significantly different. These values were similar to those obtained by Ullrey et al. (1960a). Mean corpuscular volume increased significantly ($P<0.001$) with increasing iron levels on both protein treatments. Furthermore, the values for pigs receiving the soy diets were significantly ($P<0.03$) greater than those of pigs receiving casein. Differences in reticulocyte counts were not significant, although for each treatment, final values were lower than initial values.

Serum iron values and other related parameters are shown in table 6. Regardless of the protein source, serum iron values increased with increasing levels of supplemental iron ($P<0.001$). However, there were no significant differences found between treatment means with reference to total and unbound iron-binding capacity. Transferrin saturation decreased with decreasing dietary iron ($P<0.001$). Consequently, the more anemic animals had the lowest transferrin saturation.

Results of the serum protein analyses are presented in table 7. Increasing dietary iron levels resulted in increasing α-globulin levels ($P<0.05$).

The balance data from trial I are shown in table 8 and figure 1. Chemical analysis of the water used in the trial indicated the presence of no measurable amounts of iron. Consequently water consumption data were not included in the table. Significant differences were observed in food intake. This was a consequence of the extremely low intake of the pigs receiving the 45 ppm casein diet. Differences in the daily iron intake were also significant ($P<0.001$) due to the higher amounts

Table 6. Serum iron, total and unbound iron-binding capacity and transferrin saturation, trial 1.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	45	88	152	73	137	189	±SE[1]	Prot.	Iron	Repl.	Prot.xIron
Serum iron, mcg/100 ml serum											
Initial	147	190	144	133	154	155	15.34	0.31	0.13	0.07	0.34
Final	119	155	195[bb]	127	155	179[b]	13.59	0.82	0.00	0.29	0.66
TIBC, mcg/100 ml serum											
Initial	198	271	247	224	209	292	28.51	0.89	0.16	0.03	0.17
Final	213	219	261	192	228	237	20.08	0.49	0.10	0.49	0.68
UIBC, mcg/100 ml serum											
Initial	51	81	103	91	55	137	53.34	0.62	0.48	0.31	0.60
Final	94	66	66	65	73	59	11.56	0.31	0.36	0.73	0.32
Transferrin saturation, %											
Initial	74.5	71.1	58.7	59.0	74.0	58.7	5.80	0.39	0.09	0.16	0.27
Final	56.0	71.5[a]	74.9[aa]	66.8	68.9[a]	75.0[aa]	4.06	0.42	0.01	0.79	0.25

[1] Standard error of the mean.
[a] Significantly greater than least value ($P<0.05$); [aa] ($P<0.01$).
[b] Significantly greater than least two values ($P<0.05$); [bb] ($P<0.01$).

Table 7. Serum protein analyses, trial 1.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	45	88	152	73	137	189	±SE[1]	Prot.	Iron	Repl.	Prot.xIron
Total serum protein, g/100 ml serum											
Initial	6.7	6.4	6.8	6.6	6.7	6.2	0.28	0.67	0.85	0.22	0.27
Final	6.9	7.0	7.3	7.0	6.2	6.4	0.59	0.27	0.85	0.35	0.68
Serum albumin, % of total serum protein											
Initial	32.8	38.0	35.2	32.2	37.7	34.8	1.94	0.78	0.05	0.63	0.99
Final	42.4	46.5	40.4	48.7	50.0	44.0	3.94	0.19	0.34	0.81	0.92
Serum α-globulin, % of total serum protein											
Initial	30.3	27.2	27.8	30.4	28.4	32.5	1.49	0.12	0.19	0.32	0.30
Final	27.0	30.2	32.1	29.5	27.3	33.1[b]	1.74	0.91	0.05	0.64	0.32
Serum β-globulin, % of total serum protein											
Initial	13.8	14.5	14.6	11.8	12.4	13.9	1.09	0.09	0.44	0.42	0.75
Final	14.2	13.4	12.7	12.1	12.1	11.7	0.78	0.04	0.45	0.60	0.78
Serum γ-globulin, % of total serum protein											
Initial	23.1	20.9	24.3	21.2	22.3	18.1	2.13	0.22	0.90	0.36	0.23
Final	9.6	9.9	14.1	9.8	10.6	11.3	1.71	0.64	0.21	0.31	0.56

[1] Standard error of the mean.
[b] Significantly greater than least two values ($P<0.05$).

Table 8. Balance data, trial 1.

Protein source	Casein			Soy				Significance (P value)		
Iron level, ppm	45	88	152	73	137	189	±SE[1]	Prot.	Iron	Prot.xIron
Daily food intake, g[2]	150	229[aa]	250[aa]	228[aa]	250[aa]	242[aa]	9.05	0.01	0.00	0.01
Fe balance, daily										
Fe intake, mg	6.8	20.1[aa,b]	38.0[cc,d]	16.7[aa]	34.3[cc]	46.3[ee]	0.87	0.00	0.00	0.05
Fecal Fe, mg	4.2	8.9	12.6	12.9	10.6	20.3[c]	2.44	0.05	0.05	N.S.
Urinary Fe, mg	0.4	0.1	0.4	0.4	0.4	0.4	0.09	N.S.	N.S.	N.S.
Fe retention, mg	2.2	11.1	25.3[bb,c]	3.4	23.3[bb,c]	25.6[bb,c]	2.76	N.S.	0.00	N.S.
Fe retention, %	33	54	67[a]	20	68[a]	55	12.13	N.S.	0.05	N.S.

1 Standard error of the mean.
2 Two pigs per treatment.
[a] Significantly greater than least value ($P<0.05$); [aa] ($P<0.01$).
[b] Significantly greater than least two values ($P<0.05$); [bb] ($P<0.01$).
[c] Significantly greater than least three values ($P<0.05$); [cc] ($P<0.01$).
[d] Significantly greater than least four values ($P<0.05$); [dd] ($P<0.01$).
[e] Significantly greater than all other values ($P<0.05$); [ee] ($P<0.01$).

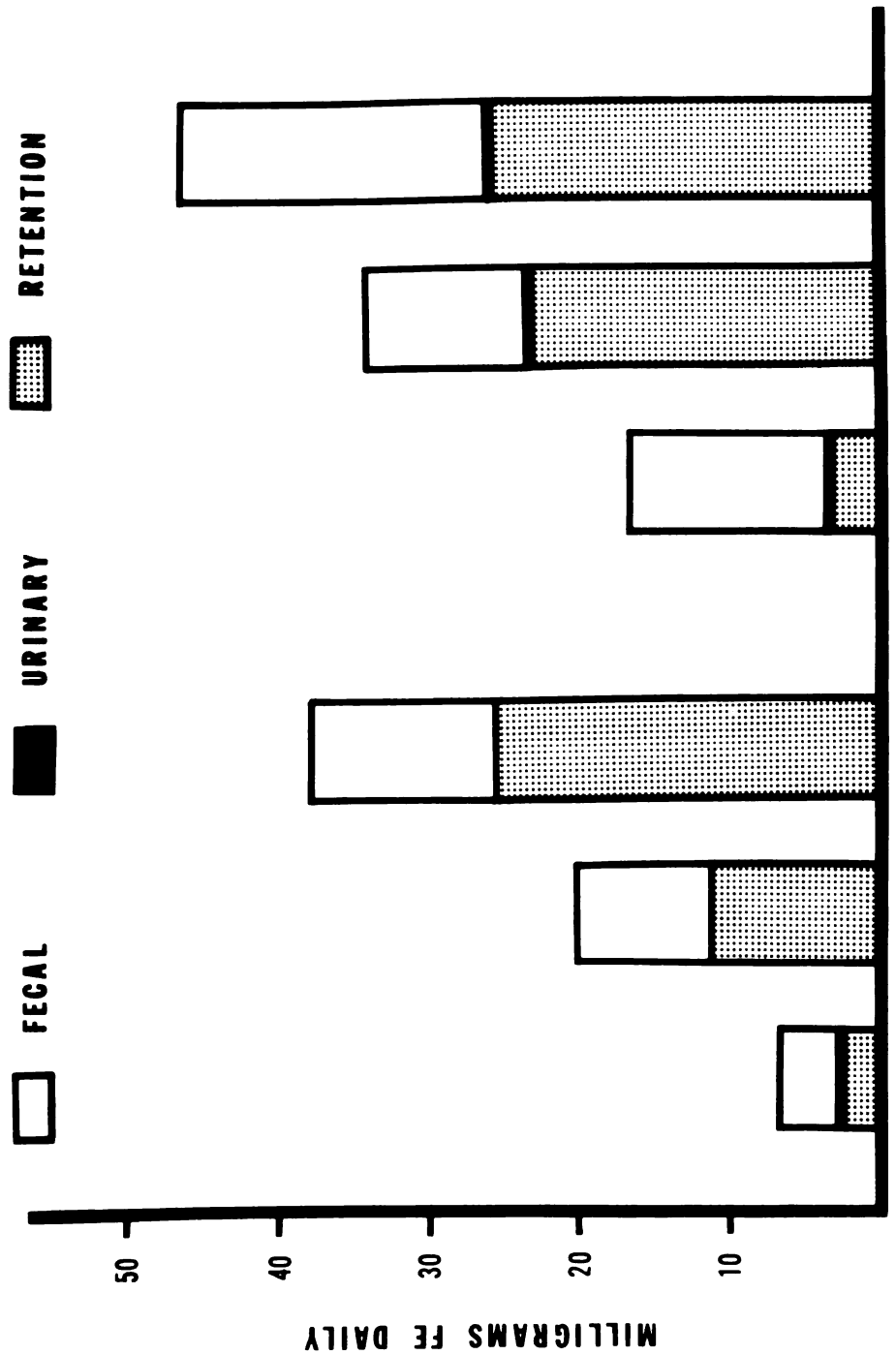

Figure 1. Effect of dietary protein source and iron level on iron balance, trial 1.

of iron in the three soy protein diets. Fecal iron increased with increasing iron levels ($P<0.05$). Also, fecal iron output was greater ($P<0.05$) on the soy diets than on the casein.

The retention data are difficult to interpret. In general, anemic animals retain more iron, on a percentage basis, than normal animals. In this instance, however, pigs receiving the basal soy and casein diets retained less iron on a percentage basis than pigs on the other four treatments. Hendricks (1967) carried out iron balance studies with baby pigs receiving 40% of either casein or soy as the protein source. Percent iron retention on the casein diet which contained 106 ppm of iron, was 35%. Retention on the soy diet (194 ppm of iron) was 50%. These values are somewhat different from those given in table 8. Because of the unequal iron levels in the different diets it is difficult to make a definite statement in relation to the effect of the protein source on iron absorption and utilization.

Some conclusions may be drawn from data of pigs on the 152 ppm casein and the 137 ppm soy diets, as these two diets approached equivalence in terms of iron content. No significant differences were observed in final weight or hematocrit, hemoglobin and erythrocyte values. Also the iron retention percentages for the two treatments were almost identical. Consequently it can be stated that under the conditions of the trial, at the level of iron supplementation mentioned above, the protein source had no significant effect on iron absorption and utilization.

Trial 2

Pig performance data are shown in table 9. Average daily gain increased with increasing levels of dietary iron ($P<0.05$) and

Table 9. Summarized pig performance data, trial 2.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	101	148	189	95	147	189	±SE[1]	Prot.	Iron	Repl.	Prot.xiron
Initial wt., kg	2.7	2.7	2.6	2.7	2.7	2.7	0.34	0.92	0.99	0.46	0.99
Final wt., kg	7.6	7.6	9.9	8.9	9.7	10.4	1.02	0.15	0.19	0.72	0.70
Avg daily gain, kg	0.14	0.14	0.21[b]	0.18	0.20	0.23[bb]	0.02	0.06	0.05	0.67	0.73
Avg daily feed, kg	0.23	0.23	0.27	0.27	0.29	0.31	0.03	0.09	0.47	0.47	0.86
Gain/good	0.60	0.60	0.77[b]	0.66	0.70	0.77[b]	0.04	0.12	0.01	0.43	0.50

[1] Standard error of the mean.

[b] Significantly greater than least 2 values ($P<0.05$); [bb] ($P<0.01$).

differences between protein source approached significance ($P<0.06$). This growth difference was a consequence of higher food intake by pigs on the soy diets. This difference approached significance ($P<0.09$). It is possible that the higher intake of the soy diets was related to the palatability and physical texture of the two diets. The casein diets tended to adhere to the feeder and the sides of the pens more readily than the soy diets. Thus the more adhesive nature of the casein diets may have been a factor in reducing the intake of these diets. Feed efficiency also increased with increasing iron levels ($P<0.01$).

As can be seen from table 10, final hematocrit and hemoglobin values were significantly different and reflected changes in the iron content of the diets. Pigs on the soy diets had significantly higher hematocrit and hemoglobin values ($P<0.01$) than pigs receiving the casein diets. The final hemoglobin values in the second trial were somewhat lower than those observed in the first trial, even though the iron levels of the three casein diets had been increased. These low final values may have been due in part to the fact that the initial hematocrit and hemoglobin values of pigs on trial 2 were lower than those of pigs on trial 1, and consequently these pigs were more anemic and would require more iron to replete their tissues. Furthermore, in both trials, supplemental iron was provided as $FeSO_4.2H_2O$. Both Matrone *et al.* (1960 and Ullrey *et al.* (1960a) used $FeSO_4.7H_2O$ to provide supplemental iron. These workers also fed a liquid diet, whereas a solid diet was used in this study. It is possible that the iron supplied as $FeSO_4.2H_2O$ was in a less available form as compared to $FeSO_4.7H_2O$. Consequently, less iron would be available for absorption and utilization.

Table 10. Hematological data, trial 2.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	101	148	189	95	147	189	±SE[1]	Prot.	Iron	Repl.	Prot.xIron
Hematocrit, %											
Initial	25.3	23.9	24.7	27.9	24.9	24.4	1.68	0.44	0.37	0.01	0.71
Final	22.0	21.1	25.3	23.2	25.6	33.7[ee]	1.85	0.01	0.01	0.28	0.18
Hemoglobin, g/100 ml blood											
Initial	7.4	7.1	7.6	8.2	7.4	6.9	0.53	0.73	0.55	0.02	0.36
Final	5.2	4.9	7.1[a]	5.7	6.5	9.9[ee]	0.65	0.01	0.00	0.22	0.24
Mean corpuscular hemoglobin concentration, %											
Initial	29.1	29.6	30.1	29.4	29.7	28.3	0.55	0.12	0.70	0.72	0.03
Final	23.5	23.4	28.1[b]	24.4	25.5	29.2[c]	0.91	0.08	0.00	0.31	0.80
Erythrocytes, $10^6/mm^3$											
Initial	4.42	4.29	4.60	4.76	4.70	4.53	0.28	0.35	0.95	0.20	0.67
Final	5.38	5.31	5.45	5.90	6.63[c]	6.74[c]	0.35	0.00	0.43	0.55	0.45
Mean corpuscular volume, cubic microns											
Initial	57.4	55.6	53.9	58.8	53.3	53.4	3.58	0.87	0.44	0.42	0.87
Final	40.8	39.7	46.6	39.2	38.9	50.7[d]	2.67	0.79	0.01	0.09	0.52
Reticulocytes, % of erythrocytes											
Initial	1.8	3.0	2.3	3.0	2.3	3.0	0.57	0.41	0.85	0.04	0.26
Final	3.8[b]	2.2	3.0	4.0[c]	2.0	1.9	0.52	0.37	0.01	0.09	0.43

[1] Standard error of the mean.
[a] Significantly greater than least value ($P<0.05$).
[b] Significantly greater than least two values ($P<0.05$).
[c] Significantly greater than least three values ($P<0.05$).
[d] Significantly greater than least four values ($P<0.05$).
[e] Significantly greater than all other values ($P<0.05$); [ee] ($P<0.01$).

Mean corpuscular hemoglobin concentration increased with increasing levels of dietary iron ($P<0.001$). Erythrocyte counts from pigs receiving soy diets were significantly ($P<0.002$) greater than those from pigs receiving casein diets. Mean corpuscular volume increased with increasing dietary iron levels ($P<0.01$), whereas reticulocyte counts decreased with increasing dietary iron levels ($P<0.01$).

Serum iron values and other related parameters are shown in table 11. Serum iron values increased with increasing levels of dietary iron ($P<0.01$). Total iron-binding capacity also increased with increasing dietary iron levels ($P<0.002$). Anemic animals usually have higher TIBC values than normal animals (Ullrey et al., 1960a). In this instance, the reverse occurred. As in trial 1, percent transferrin saturation decreased with decreasing dietary iron levels, although differences were not significant. The transferrin saturation values were smaller than those in trial 1, again a reflection of the low iron status of the pigs in trial 2.

Data from the serum protein analyses are presented in table 12. Serum albumin levels increased with increasing dietary iron level ($P<0.02$) whereas serum α-globulin levels decreased ($P<0.02$). Although there was no significant effect of iron level on β-globulin levels, there was a negative linear effect of dietary iron on the transferrin-containing β-globulin fraction in both trials.

Data from the balance trial are shown in table 13 and figure 2. The daily iron intake reflected the different iron content of the diets ($P<0.001$). Fecal iron levels increased with increasing dietary iron levels ($P<0.05$) and fecal iron output was greater from pigs receiving the casein diets. Iron retention increased with

Table 11. Serum iron, total and unbound iron-binding capacity and transferrin saturation, trial 2.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	101	148	189	95	147	189	±SE[1]	Prot.	Iron	Repl.	Prot.xIron
Serum iron, mcg/100 ml serum											
Initial	61	75	45	56	62	80	13.47	0.63	0.76	0.20	0.20
Final	94	89	184	97	98	212[d]	31.47	0.61	0.01	0.46	0.91
TIBC, mcg/100 ml serum											
Initial	191	238	210	200	195	226	23.54	0.76	0.57	0.48	0.41
Final	174	198	286	194	175	319[bb,d]	30.86	0.70	0.00	0.25	0.64
UIBC, mcg/100 ml serum											
Initial	130	163	166	145	133	147	26.50	0.61	0.78	0.13	0.68
Final	80	109	102	97	77	107	15.00	0.78	0.56	0.19	0.27
Transferrin saturation, %											
Initial	35.9	31.9	20.7	31.9	31.2	37.1	5.80	0.56	0.83	0.17	0.42
Final	54.6	46.0	59.3	50.4	56.4	65.9	4.06	0.40	0.16	0.68	0.48

[1] Standard error of the mean.
[b] Significantly greater than least value ($P<0.05$); [bb] ($P<0.01$).
[d] Significantly greater than least four values ($P<0.05$).

Table 12. Serum protein analyses, trial 2.

Protein source	Casein			Soy				Significance (P value)			
Iron level, ppm	101	148	189	95	147	189	±SE[1]	Prot.	Iron	Repl.	Prot.xIron
Total serum protein, g/100 ml serum											
Initial	6.7	6.5	6.7	6.9	7.4	6.7	0.30	0.14	0.71	0.88	0.30
Final	6.7	6.3	6.0	6.5	6.3	6.3	0.16	0.85	0.06	0.28	0.34
Serum albumin, % of total serum protein											
Initial	30.8	30.1	27.3	29.5	30.2	28.7	2.29	0.97	0.57	0.67	0.84
Final	42.3	42.8	46.9	41.6	41.9	46.6	1.65	0.65	0.02	0.43	0.98
Serum α-globulin, % of total serum protein											
Initial	30.7[a]	29.7	31.2[a]	27.4	28.5	30.6[a]	0.90	0.04	0.10	0.54	0.30
Final	32.3[a]	30.7	28.2	31.5	33.1[b]	28.8	1.22	0.50	0.02	0.47	0.46
Serum β-globulin, % of total serum protein											
Initial	12.4	12.5	12.9	13.0	12.3	12.4	0.67	0.90	0.86	0.83	0.69
Final	14.3	13.8	12.9	13.8	13.5	11.7	0.76	0.34	0.08	0.54	0.83
Serum γ-globulin, % of total serum protein											
Initial	26.0	27.8	28.7	29.1	29.1	28.2	2.04	0.45	0.88	0.68	0.70
Final	11.2	12.7	12.1	13.1	11.5	12.9	0.99	0.54	0.92	0.72	0.31

[1] Standard error of the mean.
[a] Significantly greater than least value ($P<0.05$).
[b] Significantly greater than least two values ($P<0.05$).

Table 13. Balance data, trial 2.

Protein source	Casein			Soy				Significance (P value)		
Iron level, ppm	101	148	189	95	147	189	±SE[1]	Prot.	Iron	Prot.xIron
Daily food intake, g[2]	196	220	236	220	235	235	17.84	N.S.	N.S.	N.S.
Fe balance, daily										
Fe intake, mg	19.7	32.5[bb]	44.6[cc,d]	20.8	34.5[bb]	44.2[dd]	1.84	N.S.	0.00	N.S.
Fecal Fe, mg	10.0	16.7[a]	21.2[aa,d]	5.5	10.5	10.2	2.37	0.05	0.05	N.S.
Urinary Fe, mg	0.1	0.1	0.1	0.1	0.1	0.1	0.03	N.S.	N.S.	N.S.
Fe retention, mg	9.6	15.7[a]	23.3[aa,c]	15.2[a]	23.9[bb,c]	33.9[ee]	1.51	0.00	0.00	N.S.
Fe retention, %	49	48	52	73[b]	69	77[c]	5.89	0.01	N.S.	N.S.

1 Standard error of the mean.
2 Two pigs per treatment.
a Significantly greater than least value (P<0.05); aa (P<0.01).
b Significantly greater than least two values (P<0.05); bb (P<0.01).
c Significantly greater than least three values (P<0.05); cc (P<0.01).
d Significantly greater than least four values (P<0.05); dd (P<0.01).
e Significantly greater than all other values (P<0.05); ee (P<0.01).

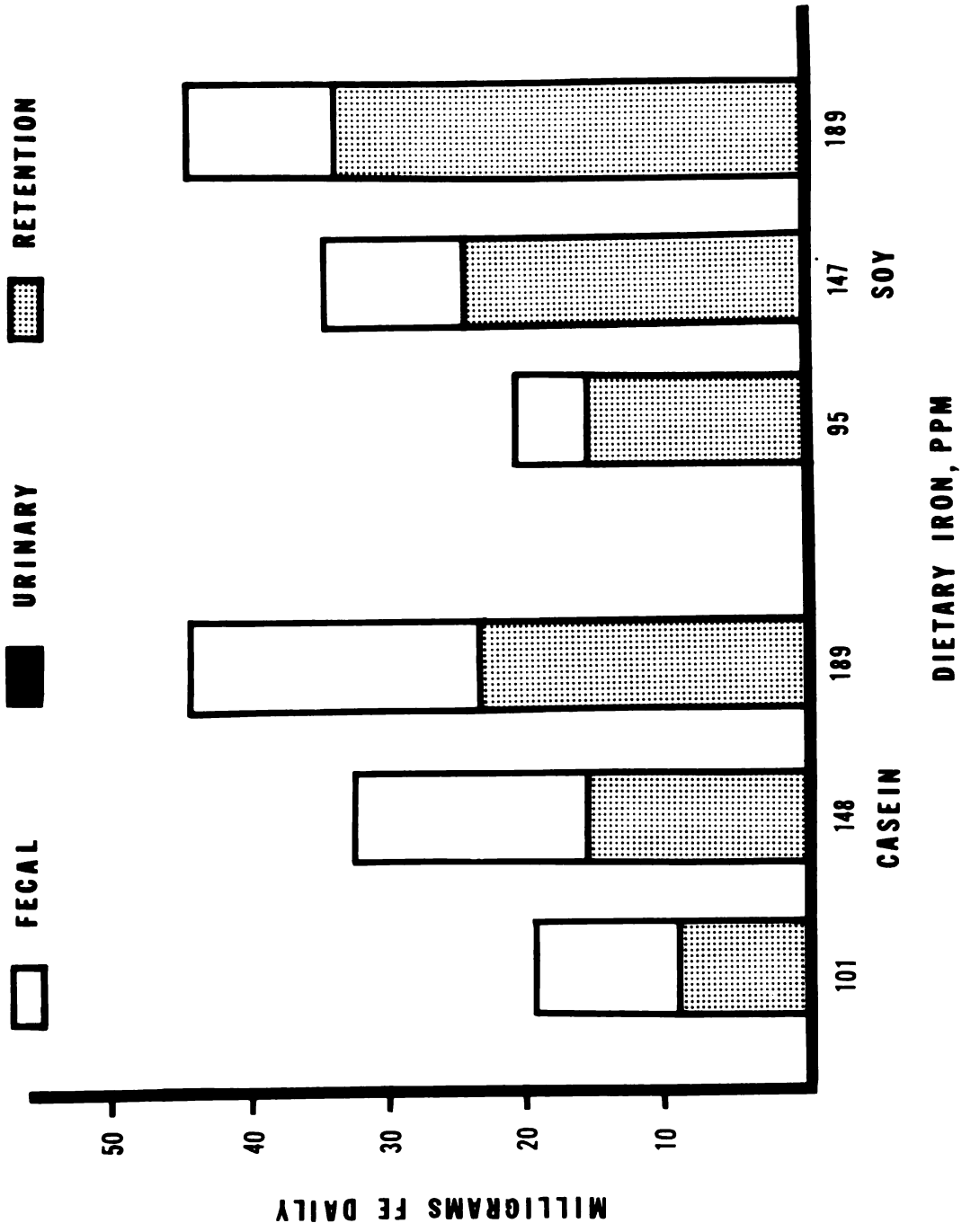

Figure 2. Effect of dietary protein source and iron level on iron balance, trial 2.

increasing dietary iron levels ($P<0.001$) and was also significantly greater in pigs receiving the soy diets. On a percentage basis, iron retention was significantly greater ($P<0.001$) for pigs receiving the soy diets. The percentage retention figures for pigs receiving the casein diets seem rather low especially in view of the anemic nature of these pigs, and an explanation is not readily forthcoming. Hendricks (1967) noted that pigs receiving a 40% casein diet containing 106 ppm of iron retained 35% of their iron intake, whereas pigs receiving a 40% soy diet containing 194 ppm of iron retained 50% of their iron intake. In this instance also, pigs receiving the higher level of iron were retaining more iron on a percentage basis. Hendricks further stated that: "the rapidly growing baby pig adapts well to limited dietary alterations, therefore, single short term mineral balance trials are not always reliable as a measure of mineral utilization during an entire experimental period".

As judged by growth and balance data, hematocrit, hemoglobin and erythrocyte values, the soy protein diets proved superior to the casein diets. Certainly it would appear from the data presented that the iron present in the soy protein is at least equally, if not more, available than that in the casein. The higher values on the soy diets may have been due, in part, to the increased food intake and consequently increased iron intake of these pigs.

Davis et al. (1968) concluded that for chicks, the iron in soybean protein was as available as that in casein. Fritz (1969) stated that the iron in isolated soy protein was as available as that in ferrous gluconate, ferrous fumarate and ferric ammonium citrate for hemoglobin

regeneration in rats and chickens. From the data presented, it would appear that for the baby pig, the iron in isolated soy is more available than that in casein.

V. CONCLUSIONS

Under the experimental conditions employed, the following conclusions can be made.

1. As judged by balance data, hemoglobin, hematocrit, erythrocyte and serum iron levels, the iron in isolated soy protein was more available than that in high protein casein. Consequently, the iron requirement of the baby pig fed soy protein diets is less than that of baby pigs fed casein diets.
2. The performance of pigs receiving the soy diets was significantly superior to that of pigs receiving the casein diets. However, by hematological standards, most of the pigs were anemic.
3. In view of the low final hemoglobin and hematocrit values observed in trial 2, some doubt must be cast as to the efficacy of $FeSO_4.2H_2O$ as a source of dietary supplemental iron for baby pigs fed synthetic diets.

VI. SUMMARY

Two trials involving a total of 48 baby pigs, were conducted to study the effect of source of protein, isolated soy or high protein casein, on iron utilization by the baby pig. Three levels of supplemental iron were used: 0, 50 and 100 ppm.

Trial 1

Twenty-four baby pigs were assigned, 4 per lot, to the following dietary treatments: basal casein, basal isolated soy protein, basal casein plus 50 ppm supplemental iron, basal isolated soy protein plus 50 ppm supplemental iron, basal casein plus 100 ppm supplemental iron, or basal isolated soy protein plus 100 ppm supplemental iron. It was intended that the total iron content of the soy and casein diets should have been equal at each of the three levels of iron supplementation. However, it was found that the analytical iron content of the isolated soy protein was greater than the assumed value. Consequently, the total iron content of the three soy diets was greater than that of the respective casein diets. In view of this, it was not possible to make a valid comparison of the effect of the protein source on iron utilization by the baby pig.

Trial 2

Twenty-four baby pigs were again assigned, 4 pigs per lot, to treatments as in the first trial. In trial 2, however, the supplemental iron levels of the casein diets were increased so that the total iron content of the casein and soy diets was equal at each level of

iron supplementation. As analysed the iron content of the casein diets was 101, 148 and 189 ppm and for the soy diets 95, 147 and 189 ppm. As judged by growth and balance data, hematocrit, hemoglobin and erythrocyte values, the iron in the soy diets was more available than that in high protein casein diets. Consequently, under the conditions of the experiment, it appears that the iron requirement of the baby pig fed isolated soy protein is less than that of pigs receiving casein as the protein source.

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APPENDIX

Appendix Table 1. Mineral mixtures used in experimental diets.

	Basal		Basal +50 ppm Fe		Basal+100 ppm Fe	
	Casein	Soy	Casein	Soy	Casein	Soy
	g		g		g	
KCl	100	100	100	100	100	100
KI	0.02	0.02	0.02	0.02	0.02	0.02
$CuSO_4$	1	1	1	1	1	1
$MnSO_4$	1	1	1	1	1	1
$CoCO_3$	1	1	1	1	1	1
$MgCO_3$	20	20	20	20	20	20
$NaHCO_3$	250	250	250	250	250	250
$CaHPO_4.2H_2O$	360	360	360	360	360	360
$CaCO_3$	125	125	125	125	125	125
$ZnSO_4.H_2O$	4	4	4	4	4	4
$FeSO_4.2H_2O$[a]	1.67	0	4.45	2.78	7.23	5.56
Cerelose[a]	136.31	137.98	133.53	135.20	130.75	132.42
$FeSO_4.2H_2O$[b]	3.32	0	6.10	2.78	8.88	5.56
Cerelose[b]	134.66	137.98	131.88	135.20	129.10	132.42

[a] Trial 1.
[b] Trial 2.

Appendix Table 2. Vitamin mixture used in experimental diets.

	ppm in diet
Thiamine mononitrate	3
Riboflavin	6
Nicotinamide	40
Calcium pantothenate	30
Pyridoxine hydrochloride	2
Para amino benzoic acid	13
Ascorbic acid	80
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

Appendix Table 3. Pig performance data, trial 1.

Treatment	Pig No.	Weight, Kg					Av.Da. Gain,g	Av.Da. Feed,g	Gain/ Feed
		Init.	2 wt	3 wt	4 wt	5 wt			
45 ppm Fe Casein	Y17-6	1.90	2.30	2.96	3.68	4.42	87	141	0.62
	H29-2	2.42	2.72	2.90	3.14	3.54	39	163	0.24
	Y17-9	2.08	2.64	3.42	4.90	6.06	137	200	0.69
73 ppm Fe Soy	Y17-8	1.70	2.90	4.48	5.34	6.66	171	216	0.79
	Y57-9	2.46	3.16	4.60	5.70	7.04	158	211	0.75
	Y17-7	2.20	3.22	4.98	6.92	10.14	274	304	0.90
	H29-4	1.90	2.30	2.44	2.90	3.48	54	168	0.32
88 ppm Fe Casein	Y17-2	1.98	2.52	3.70	4.88	6.82	167	246	0.68
	Y57-12	1.65	2.44	3.88	4.68	6.24	158	188	0.84
	Y17-11	2.01	2.96	5.20	7.90	11.46	326	360	0.91
	Y57-2	2.40	2.60	3.36	4.70	7.10	162	181	0.89
137 ppm Fe Soy	Y17-3	2.18	3.60	5.88	6.46	10.22	277	281	0.99
	Y57-3	2.66	4.00	6.06	6.76	9.54	237	270	0.88
	H29-5	1.82	1.84	2.34	3.08	3.80	68	199	0.34
	Y57-14	1.62	2.22	2.86	4.24	6.30	161	186	0.87
152 ppm Fe Casein	Y17-4	2.10	3.10	5.00	6.12	9.26	247	283	0.87
	Y57-10	2.08	3.06	4.76	5.86	8.72	229	282	0.81
	H29-1	2.38	2.52	2.80	3.40	3.70	46	172	0.27
	Y57-4	1.96	2.22	3.48	5.16	7.90	205	270	0.76
189 ppm Fe Soy	Y17-5	2.00	2.92	5.04	5.76	8.32	218	263	0.83
	Y57-13	2.18	3.24	5.04	5.86	9.80	263	218	0.90
	Y17-1	2.88	4.50	7.54	10.72	14.12	388	503	0.77
	Y57-6	1.32	1.60	2.30	4.00	5.52	145	209	0.69

Appendix Table 4. Hematological data, trial 1.

Treatment	Pig No.	Hemoglobin, g/100 ml blood		Hematocrit %		Mean Corpuscular Hemoglobin Conc.		Erythrocytes, $10^6/mm^3$		MCV[a]		Reticulocytes[b]	
		Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
45 ppm Fe	Y17-6	5.19	4.76	20.5	18.5	25.3	25.7	3.87	5.95	53.0	31.1	1.7	3.6
Casein	H29-2	7.73	6.82	28.4	25.9	27.2	26.3	2.57	7.10	110.5	36.5	3.0	1.5
	Y17-9	9.10	4.72	31.9	18.0	28.5	26.2	5.58	5.20	57.2	34.6	2.9	0.8
73 ppm Fe	Y17-8	8.67	7.60	32.1	27.2	27.0	27.9	5.87	6.62	54.7	41.1	1.2	1.1
Soy	Y57-9	8.58	7.25	29.7	24.0	28.9	30.2	4.89	6.75	60.7	35.6	2.5	2.0
	Y17-7	8.93	8.15	31.9	28.9	28.0	28.2	5.56	6.57	57.4	44.0	2.2	1.5
	H29-4	8.46	6.74	31.3	25.2	27.0	26.7	2.54	6.99	123.2	36.1	2.7	2.2
88 ppm Fe	Y17-2	8.88	9.10	30.0	30.2	29.6	30.1	5.05	7.02	59.4	43.0	1.9	1.0
Casein	Y57-12	8.07	7.64	27.0	26.0	29.9	29.4	4.44	7.71	60.8	33.7	1.7	2.1
	Y17-11	9.79	9.66	34.8	32.6	28.1	29.6	5.45	6.52	63.9	50.0	2.4	1.2
	Y57-2	6.65	7.08	22.8	27.1	29.2	26.1	3.72	7.94	61.3	34.1	4.6	1.2
137 ppm Fe	Y17-3	9.83	9.36	33.8	28.8	29.1	32.5	5.45	5.69	62.0	50.6	2.2	0.6
Soy	Y57-3	7.85	10.04	25.1	34.4	31.3	29.2	3.52	6.97	71.3	49.4	3.9	1.2
	H29-5	7.51	6.05	28.1	27.0	26.7	22.4	2.16	6.94	130.1	38.9	3.9	2.1
	Y57-14	5.92	7.60	21.1	27.0	28.1	28.1	3.83	5.43	55.1	49.7	3.7	1.4
152 ppm Fe	Y17-4	7.25	10.17	26.7	34.8	27.2	29.2	4.57	6.39	58.4	54.5	2.8	0.9
Casein	Y57-10	8.03	8.88	26.3	31.5	30.5	28.2	3.95	6.81	66.6	46.3	1.0	2.7
	H29-1	6.70	7.60	27.3	30.7	24.5	24.8	2.83	8.23	96.3	37.3	2.4	1.9
	Y57-4	4.81	9.78	19.5	34.8	24.7	28.1	2.14	6.29	91.1	55.3	6.1	3.9
189 ppm Fe	Y17-5	8.54	11.50	31.4	35.9	27.2	32.0	5.47	7.59	57.4	47.3	2.9	0.9
Soy	Y57-13	6.40	7.90	23.9	32.1	26.8	24.6	3.68	5.02	64.9	63.9	4.7	0.8
	Y17-1	7.25	11.16	25.4	36.8	28.5	30.3	4.56	7.12	55.7	51.7	1.2	0.8
	Y57-6	7.68	7.81	26.8	32.8	28.7	23.8	4.05	5.15	66.2	63.7	5.5	3.2

a Mean corpuscular volume, cubic microns.

b Percent of erythrocytes.

Appendix Table 5. Serum iron, total and unbound iron-binding capacity and transferrin saturation data, trial 1.

Treatment	Pig No.	Serum Iron[a]		TIBC[a]		UIBC[a]		Transferrin Saturation %	
		Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
45 ppm Fe Casein	Y17-6	156	129	192	206	36	77	81.3	62.6
	H29-2	143	118	224	223	81	105	63.8	52.9
	Y17-9	141	110	179	209	38	99	78.8	52.6
73 ppm Fe Soy	Y17-8	150	118	235	146	85	28	63.8	80.8
	Y57-9	123	125	229	191	106	66	35.6	28.9
	Y17-7	121	169	203	248	82	78	53.7	65.4
	H29-4	136	97	230	184	94	87	59.1	52.7
88 ppm Fe Casein	Y17-2	138	150	176	227	38	77	78.4	66.1
	Y57-12	239	100	361	131	122	31	66.2	76.3
	Y17-11	137	207	200	299	63	92	68.5	69.2
	Y57-2	246	162	345	218	99	57	71.3	74.3
137 ppm Fe Soy	Y17-3	129	159	224	262	95	103	57.6	60.7
	Y57-3	146	168	196	264	50	96	74.5	63.6
	H29-5	154	121	209	180	55	59	73.7	67.2
	Y57-14	188	172	208	205	20	33	90.4	83.9
152 ppm Fe Casein	Y17-4	97	206	189	290	92	84	51.3	71.0
	Y57-10	159	173	279	248	120	75	57.0	69.8
	H29-1	173	197	225	261	52	64	76.9	75.5
	Y57-4	146	204	294	245	148	41	49.7	83.3
189 ppm Fe Soy	Y17-5	110	179	227	237	117	58	48.5	75.5
	Y57-13	155	155	462	227	307	72	33.5	68.3
	Y17-1	142	224	167	273	25	49	85.0	82.1
	Y57-6	211	157	311	212	100	55	67.8	74.1

[a] mcg/100 ml serum.

Appendix Table 6. Serum protein analyses, trial 1.

Treatment	Pig No.	Total Serum Protein[a]		Serum Albumin[b]		Serum α-globulin[b]		Serum β-globulin[b]		Serum γ-globulin[b]	
		Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
45 ppm Fe Casein	Y17-6	6.2	6.3	33.1	29.4	31.8	29.4	15.6	14.5	19.5	6.4
	H29-2	7.2	7.9	31.1	52.6	26.3	22.9	11.6	13.0	31.1	11.5
	Y17-9	6.6	6.5	34.2	45.1	32.9	28.8	14.2	15.2	18.7	11.0
73 ppm Fe Soy	Y17-8	5.5	7.0	34.4	44.8	33.9	34.1	12.9	13.6	18.8	7.5
	Y57-9	6.3	7.9	36.8	50.0	29.6	26.1	14.0	13.5	19.6	10.4
	Y17-7	7.3	6.2	33.5	61.6	33.6	25.8	12.0	7.6	21.0	5.1
	H29-4	7.2	6.7	23.9	38.3	24.6	31.9	8.4	13.8	25.2	16.0
88 ppm Fe Casein	Y17-2	6.6	8.5	34.7	46.3	28.2	30.3	15.6	12.7	21.4	10.6
	Y57-12	6.1	7.7	41.3	43.5	24.5	33.0	13.6	13.5	23.2	10.0
	Y17-11	5.7	5.5	35.6	47.7	30.6	28.5	14.2	13.0	19.6	10.9
	Y57-2	7.0	6.2	40.3	48.5	25.6	29.1	14.7	14.3	19.3	8.0
137 ppm Fe Soy	Y17-3	6.0	8.9	39.2	60.3	31.2	23.9	13.2	10.1	19.6	5.6
	Y57-3	7.2	5.9	42.0	51.2	25.8	26.5	11.7	13.0	20.3	9.2
	H29-5	7.3	5.1	34.5	42.0	25.1	29.3	11.1	13.2	29.2	15.5
	Y57-14	6.4	4.9	35.1	46.4	31.4	29.6	13.4	12.0	20.1	12.0
152 ppm Fe Casein	Y17-4	6.3	7.1	36.0	42.7	30.5	33.1	14.6	12.3	18.9	11.8
	Y57-10	6.1	5.4	37.8	45.1	26.9	32.0	11.5	12.1	23.7	11.2
	H29-1	7.5	8.0	28.8	32.0	25.5	31.1	11.8	12.0	34.0	21.7
	Y57-4	7.1	8.6	38.1	41.7	28.4	32.3	20.4	14.3	20.4	11.6
189 ppm Fe Soy	Y17-5	6.2	7.2	35.2	50.0	31.7	28.2	15.2	11.6	17.9	10.2
	Y57-3	6.3	5.9	31.0	38.3	34.3	37.8	13.5	11.4	18.5	12.4
	Y17-1	5.9	6.4	37.7	49.2	29.8	28.4	13.9	12.0	18.5	10.4
	Y57-6	6.3	5.9	35.2	38.6	34.2	37.8	13.1	11.6	17.4	12.0

[a] g/100 ml serum. [b] Percent of total serum protein.

Appendix Table 7. Balance data, trial 1.[a]

Treatment	Pig No.	Food intake, g	Fe intake, mg	Fecal Fe, mg	Urinary Fe, mg	Fe retention, mg	Fe retention, %
45 ppm Fe Casein[b]	Y17-6	150	6.75	4.17	0.38	2.20	32.59
73 ppm Fe	Y17-8	223	16.30	15.55	0.56	0.19	1.17
Soy	Y57-9	233	17.06	10.21	0.23	6.62	38.80
88 ppm Fe	Y17-2	250	22.00	7.80	0.11	14.09	64.05
Casein	Y57-12	208	18.27	9.91	0.17	8.19	44.83
137 ppm Fe	Y17-3	250	34.25	13.57	0.52	20.16	58.86
Soy	Y57-3	250	34.25	7.62	0.25	26.38	77.02
152 ppm Fe	Y17-4	250	38.00	8.43	0.13	29.44	77.47
Casein	Y57-10	250	38.00	16.76	0.08	21.16	55.68
189 ppm Fe	Y17-5	240	45.36	19.15	0.45	25.76	56.79
Soy	Y57-13	250	47.25	21.47	0.26	25.52	54.01

[a] Per day.
[b] Balance data of H29-2 not used in statistical analysis because of negative iron balance.

Appendix Table 8. Pig performance data, trial 2.

Treatment	Pig No.	Weight, Kg						Av.Da. Gain,g	Av.Da. Feed,g	Gain/ Feed
		Init.	2 wt	3 wt	4 wt	5 wt	6 wt			
101 ppm Fe Casein	Y17-2	3.84	4.32	6.62	8.10	9.02	11.74	226	336	0.67
	Y52-5	1.58	2.20	2.90	3.52	4.04	5.20	103	160	0.65
	Y6-1	2.65	3.18	4.24	4.28	5.22	6.00	96	200	0.48
95 ppm Fe Soy	Y6-4	2.44	3.30	4.40	5.18	5.84	8.00	159	225	0.71
	Y17-1	3.70	4.66	5.22	6.12	6.56	8.52	138	238	0.58
	Y50-1	1.74	1.98	2.94	3.88	6.22	9.06	209	290	0.72
	Y52-6	3.00	3.62	4.84	5.60	6.94	10.06	202	325	0.62
148 ppm Fe Casein	Y6-5	2.12	2.60	3.86	4.72	5.58	6.88	136	201	0.68
	Y17-6	2.92	2.04	3.80	4.24	5.54	7.14	121	224	0.54
	Y6-9	1.92	2.26	3.12	3.66	4.98	6.28	125	216	0.58
	Y52-1	3.68	4.22	5.90	6.52	8.34	10.00	181	295	0.61
147 ppm Fe Soy	Y6-2	2.66	3.14	4.20	4.94	6.24	9.40	193	242	0.80
	Y52-2	2.32	3.06	4.86	4.92	6.44	8.50	177	282	0.63
	Y6-8	2.28	2.66	3.74	4.60	6.48	8.82	187	282	0.66
	Y17-5	3.34	3.78	5.54	6.58	8.96	12.16	252	367	0.69
189 ppm Fe Casein	Y6-7	2.32	2.72	4.00	4.76	6.36	8.84	186	253	0.74
	Y52-3	3.28	4.26	5.90	6.32	6.94	9.98	191	263	0.73
	Y6-3	2.50	3.28	4.98	6.54	9.58	11.80	266	310	0.86
	Y17-4	2.38	3.02	4.14	4.76	6.46	9.12	193	257	0.75
189 ppm Fe Soy	Y17-3	2.82	2.62	3.96	5.42	6.92	9.94	203	266	0.76
	Y52-4	2.88	3.06	4.60	5.86	7.20	9.96	259	292	0.89
	Y6-6	2.70	4.02	6.12	7.36	10.60	13.68	314	406	0.77
	Y52-7	2.34	2.86	4.50	5.56	6.30	7.82	157	239	0.66

Appendix Table 9. Hematological data, trial 2.

Treatment	Pig No.	Hemoglobin, g/100 ml blood		Hematocrit %		Mean Corpuscular Hemoglobin Conc.		Erythrocytes, $10^6/mm^3$		MCV[a]		Reticulocytes[b]	
		Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
101 ppm Fe Casein	Y17-2	7.28	7.39	25.0	28.2	29.1	26.2	4.77	6.59	52.4	42.8	1.7	1.2
	Y52-5	7.79	4.10	26.6	18.5	29.3	22.2	4.27	4.51	62.3	41.0	1.4	6.8
	Y6-1	6.98	4.19	24.2	19.3	28.8	21.7	4.21	5.03	57.5	38.4	2.2	3.5
95 ppm Fe Soy	Y6-4	9.21	5.05	32.2	20.8	28.6	24.3	4.29	5.79	75.1	35.9	1.8	3.7
	Y17-1	6.29	4.43	21.6	19.8	29.1	22.4	3.97	5.66	54.4	35.0	1.6	5.8
	Y50-1	10.55	8.70	34.8	32.1	30.3	27.1	6.05	6.13	57.5	52.4	1.7	3.2
	Y52-6	6.72	4.76	22.8	20.0	29.5	23.8	4.72	6.00	48.3	33.3	3.7	3.4
148 ppm Fe Casein	Y6-5	7.92	5.62	27.2	22.4	29.1	25.1	4.88	5.49	55.7	40.8	1.5	2.2
	Y17-6	7.23	5.13	22.2	21.5	32.6	23.9	4.22	5.46	52.6	39.4	2.9	2.7
	Y6-9	7.23	4.72	25.6	20.8	28.2	22.7	4.36	5.21	58.7	39.9	2.4	2.4
	Y52-1	5.86	4.31	20.6	19.6	28.4	22.0	3.71	5.06	55.5	38.7	5.2	1.6
147 ppm Fe Soy	Y6-2	7.10	6.77	23.2	28.3	30.6	23.9	4.46	6.64	52.0	42.6	3.1	2.0
	Y52-2	7.62	8.13	26.2	28.2	29.1	28.8	4.27	6.68	61.4	42.2	1.1	1.5
	Y6-8	7.88	5.54	26.0	21.7	30.3	25.5	4.79	5.50	54.3	39.5	1.5	2.6
	Y17-5	6.93	5.75	24.0	24.2	28.9	23.8	5.28	7.69	45.5	31.5	3.5	1.9
189 ppm Fe Casein	Y6-7	9.47	7.67	30.0	24.8	31.6	30.9	5.42	4.92	55.4	50.4	1.2	2.3
	Y52-3	6.03	5.99	20.2	23.1	29.9	25.9	4.61	5.80	43.8	39.8	4.0	4.0
	Y6-3	8.10	7.76	26.2	27.9	30.9	27.8	4.69	5.83	55.9	47.9	1.8	2.2
	Y17-4	6.98	6.94	22.2	25.2	31.4	27.5	3.66	5.23	60.7	48.2	2.1	3.5
189 ppm Fe Soy	Y17-3	9.00	11.61	31.2	38.1	28.8	30.5	5.00	7.18	62.4	53.1	1.5	2.3
	Y52-4	5.99	9.73	21.0	33.5	28.5	29.0	4.57	7.80	46.0	42.9	3.1	2.0
	Y6-6	7.92	10.30	27.6	34.8	28.7	29.6	4.84	5.54	57.0	62.8	1.7	1.6
	Y52-7	4.82	7.88	17.8	28.3	27.1	27.8	3.69	6.42	48.2	44.1	5.7	1.5

[a] Mean corpuscular volume, cubic microns. [b] Percent of erythrocytes.

Appendix Table 10. Serum iron, total and unbound iron-binding capacity and transferrin saturation data, trial 2.

Treatment	Pig No.	Serum Iron[a]		TIBC[a]		UIBC[a]		Transferrin Saturation %	
		Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
101 ppm Fe Casein	Y17-2	42	95	238	152	196	57	17.6	62.5
	Y52-5	78	101	121	179	43	78	64.5	56.4
	Y6-1	63	87	214	191	151	104	29.4	45.5
95 ppm Fe Soy	Y6-4	75	85	147	216	72	131	51.0	39.4
	Y17-1	41	89	256	173	215	84	16.0	51.4
	Y50-1	50	113	125	198	75	85	40.0	57.1
	Y52-6	56	102	274	190	218	88	20.4	53.7
148 ppm Fe Casein	Y6-5	129	86	223	244	94	158	57.8	35.2
	Y17-6	52	84	238	168	186	84	21.8	50.0
	Y6-9	79	97	247	208	168	111	32.0	46.6
	Y52-1	39	90	244	173	205	83	16.0	52.0
147 ppm Fe Soy	Y6-2	85	53	185	121	100	68	45.9	43.8
	Y52-2	44	141	229	199	185	58	19.2	70.9
	Y6-8	95	106	214	246	119	140	44.4	43.1
	Y17-5	23	91	152	134	129	143	15.1	67.9
189 ppm Fe Casein	Y6-7	22	375	138	475	116	100	15.9	78.9
	Y52-3	65	89	241	184	176	95	27.0	48.4
	Y6-3	59	177	226	302	167	125	26.1	58.6
	Y17-4	33	94	238	183	205	89	13.9	51.4
189 ppm Fe Soy	Y17-3	130	292	191	353	61	61	68.1	82.7
	Y52-4	44	233	262	310	218	77	16.8	75.2
	Y6-6	67	153	206	296	139	143	32.5	51.7
	Y52-7	77	171	247	316	170	145	31.2	54.1

[a] mcg/100 ml serum.

Appendix Table II. Serum protein analyses, trial 2.

Treatment	Pig No.	Total Serum Protein[a]		Serum Albumin[b]		Serum α-globulin[b]		Serum β-globulin[b]		Serum γ-globulin[b]	
		Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.	Init.	Fin.
101 ppm Fe Casein	Y17-2	6.2	6.6	35.6	42.4	28.5	34.1	12.0	11.4	24.0	12.1
	Y52-5	7.1	6.8	28.5	41.7	31.7	32.0	11.5	14.7	28.2	11.7
	Y6-1	6.7	6.6	28.3	42.8	32.0	30.8	13.8	16.7	25.8	9.8
95 ppm Fe Soy	Y6-4	6.5	6.1	31.2	44.1	29.2	30.9	13.0	14.2	26.6	10.7
	Y17-1	6.7	6.7	38.4	41.8	26.1	33.3	14.8	13.6	20.7	11.4
	Y50-1	8.0	6.5	21.9	41.0	27.7	30.0	11.6	12.4	38.9	16.7
	Y52-6	6.5	6.5	26.5	39.5	26.5	31.8	12.6	15.1	30.0	13.6
148 ppm Fe Casein	Y6-5	6.9	6.5	25.6	44.0	28.9	29.7	12.8	15.8	32.8	10.4
	Y17-6	6.2	5.7	34.7	42.8	29.0	31.7	11.3	12.1	25.0	13.5
	Y6-9	6.1	6.4	28.0	40.6	31.6	32.2	12.0	13.4	28.4	13.9
	Y52-1	6.7	6.6	32.1	43.9	29.2	29.3	13.8	13.8	24.8	13.0
147 ppm Fe Soy	Y6-2	7.9	5.9	30.5	43.1	27.6	30.2	14.3	14.8	27.6	11.9
	Y52-2	7.9	6.6	24.9	36.9	30.8	38.0	11.2	11.8	33.0	13.3
	Y6-8	7.3	6.3	28.4	40.8	29.9	33.6	11.3	15.0	30.4	10.4
	Y17-5	6.5	6.5	37.0	46.8	25.5	30.4	12.2	12.5	25.2	10.4
189 ppm Fe Casein	Y6-7	6.7	5.4	27.8	50.9	28.8	24.8	13.7	12.4	29.7	11.9
	Y52-3	6.6	6.5	25.3	42.7	33.1	30.7	11.6	12.8	30.0	13.9
	Y6-3	7.1	5.9	29.4	48.7	29.4	27.4	14.7	12.8	26.5	11.1
	Y17-4	6.4	6.2	26.7	45.3	33.3	29.9	11.6	13.4	28.4	11.3
189 ppm Fe Soy	Y17-3	7.4	6.5	32.8	48.7	30.4	28.3	10.9	10.0	25.9	13.0
	Y52-4	6.5	6.2	26.1	50.2	31.6	25.5	12.4	11.2	30.0	13.1
	Y6-6	5.7	5.8	29.0	48.9	29.1	28.5	12.9	13.1	29.0	9.5
	Y52-7	7.1	6.6	27.0	38.7	31.4	32.7	13.2	12.6	28.0	15.9

[a] g/100 ml serum. [b] Percent of total serum protein.

Appendix Table 12. Balance data, trial 2.[a]

Treatment	Pig No.	Food intake, g	Fe intake, mg	Fecal Fe, mg	Urinary Fe, mg	Fe retention, mg	Fe retention, %
101 ppm Fe	Y17-2	237	23.90	13.75	0.09	10.06	42.09
Casein	Y52-5	155	15.62	6.29	0.12	9.21	58.96
95 ppm Fe	Y6-4	207	19.63	5.72	0.13	13.78	70.20
Soy	Y17-1	232	22.04	5.30	0.12	16.62	75.41
148 ppm Fe	Y6-5	228	33.74	20.48	0.17	13.09	38.80
Casein	Y17-6	211	31.18	12.86	0.08	18.24	58.50
147 ppm Fe	Y6-2	235	34.55	11.51	0.18	22.86	66.17
Soy	Y52-2	234	34.45	9.57	0.10	24.78	71.93
189 ppm Fe	Y6-7	236	44.67	23.27	0.13	21.27	47.62
Casein	Y52-3	236	44.60	19.10	0.16	25.34	56.82
189 ppm Fe	Y17-3	234	44.16	10.25	0.14	33.77	76.47
Soy	Y52-4	235	44.42	10.15	0.10	34.17	76.92

[a] Per day.