

A REVIEW OF IRON NUTRITION IN PIGS

Brian K. Anderson, MS. and Robert A. Easter, Ph.D.

Department of Animal Sciences

University of Illinois, Urbana

Man has known that iron plays an important role in health and disease for some considerable time (Loosli, 1978). In fact Bryan (1931) stated that documented therapeutic uses of iron date back as far as 1500 BC. However it was not until Boussingault (1872) that iron was recognized as a vital nutrient for animals. Braasch (1891) was credited with being the first to describe anemia in nursing pigs that were being reared in confinement in Germany. However, he did not equate the anemia with iron deficiency but instead with management. The first to link anemia in nursing pigs with iron deficiency were McGowan and Chrichton (1924). The first to realize this in the United States were Hart et al. (1929) who showed that anemia could be prevented by orally supplementing with ferrous or ferric sulfate. This was after Doyle et al. (1927) had unsuccessfully proposed that baby pig anemia was associated with reduced exposure to sunlight.

Research into bioavailability of iron in feedstuffs is limited due to the fact that anemia is of little significance in other farm species. Iron deficiency is primarily associated with nursing pigs being reared in confinement or animals dependant on milk-based diets. This limits, for practical purposes, the importance of anemia to baby or nursing pigs. Several factors which compound this susceptibility to anemia are: very low iron stores at birth, absence of the polycythemia of birth common to other animals, particularly low levels of iron in sow's milk, and very rapid growth rates compared to other species (Underwood, 1981). In fact, piglets normally quadruple their birth weight by the time they are three-weeks-of age. As mentioned earlier sow's milk is a very poor source of iron, containing only 1 to 3 parts per million (Venn et al., 1947).

The past decade has seen a marked increase in phase feeding of nursery pigs and in the utilization of by-product feed ingredients. Phase feeding is the system of feeding several diets over short periods of time to maximize gain and efficiency. Utilization of by-product ingredients has increased as the feed industry strives to find palatable, highly digestible nutrients which are economically acceptable in animal diets. Research in the area of iron bioavailability for these ingredients has been limited. However, some estimates of iron bioavailability for animal by-products range from 50-60%, with blood meals possibly being higher (Conrad et al., 1980). Hence, there is a need to further investigate the bioavailability of iron in by-product ingredients commonly used in swine diets.

This thesis will evaluate the iron bioavailability of spray-dried blood cells (SDBC) relative to ferrous sulfate in nursery pig diets. The study will consist of two separate hemoglobin (Hb) repletion experiments where hemoglobin repletion will be the criterion used in determining bioavailability.

Iron Compounds in the Body

Iron is a vital component of every living thing. Bothwell et al. (1958) estimated that a 70 kilogram adult human has a whole body iron concentration of 60 to 70 parts per million. The concentration in the pig at birth is approximately 20 to 30 parts per million (Venn et al., 1947). Of this concentration in the newborn pig, 47% is associated with blood, 1.6% is in the spleen, 15% is in liver and the remaining 44% is found in body tissues (Thoren-Tolling, 1975). Following the neonatal period around 80% of the iron in the pig is associated with hemoglobin (National Research Council, 1979). The majority of body iron is bound to proteins as heme complexes or nonheme complexes. The most common heme complexes are hemoglobin and myoglobin, while common nonheme complexes consist of two storage forms, ferritin and hemosiderin, and one transport form, transferrin.

Hemoglobin (Hb) has a molecular weight of 68,000 and consists of four atoms of iron. Hemoglobin is a tetramer consisting of four globin moieties each containing a heme unit bound loosely by noncovalent bonding of iron and the imidazole nitrogen of a histidine residue in each protein chain. The function of hemoglobin is in the transfer of oxygen from the lung to the tissues. Hemoglobin is found in the erythrocytes and makes up roughly 90% of the protein found in those cells (Davies, 1961). In a review, Zimmerman (1980) stated that hemoglobin accounts for 30% of erythrocyte weight. Hemoglobin synthesis, known as hematopoiesis, is carried on in the bone marrow. The average life span of an erythrocyte in swine is 70 days (Bush et al., 1955; Talbot and Swenson, 1963; Jensen et al., 1956; Withrow and Bell, 1969).

Myoglobin differs from hemoglobin in that it contains only one heme group. The molecular weight of myoglobin is 17,000. Myoglobin constitutes only 3 to 7% of total body iron while hemoglobin accounts for about 60% (Hahn et al., 1943). Myoglobin has a much higher affinity for oxygen than that of hemoglobin, this allows for transfer of oxygen from oxyhemoglobin to muscle cells (Fruton and Simmonds, 1958).

Ferritin is a nonheme protein and a primary storage form of body iron that can contain up to 20% iron. The iron contained in ferritin is in the form of ferric oxyhydroxide (Munro, 1977). Ferritin can be found in all tissues of the body, however it is in high concentrations in the liver, spleen, intestinal mucosa and the bone marrow.

Hemosiderin is also a nonheme protein and like ferritin is a storage form of body iron. The iron content of hemosiderin can reach 35% primarily as ferric hydroxide (Shoden and Sturgeon, 1961). Hemosiderin is thought to be produced in times of iron overload.

Transferrin is the transport form of body iron and performs the role of regulating iron distribution within the body. Transferrin like ferritin is transported to all parts of the body but is primarily transported to the liver, spleen, intestinal mucosa, and bone marrow. In swine, transferrin is dependant upon ceruloplasmin for the oxidation of ferrous iron to ferric prior to incorporation into transferrin (Lee et al., 1968; Roeser et al., 1970).

Iron Absorption

Iron absorption can occur throughout the gastrointestinal tract with the two most common sites of absorption being the duodenum and jejunum. The common theory in iron absorption, known as the mucosal block theory, is that only enough iron to meet the animal's needs is absorbed (Hahn et al., 1943). Two of the main determinants of this need are iron status and erythropoietic demand. This theory has been modified since its initial statement, however it is still believed that only a small amount of the iron a pig consumes is actually absorbed. The basis of this theory is that iron is taken up by the mucosal cells in one of three forms, ferrous, ferric or as part of an organic compound. Upon absorption ferrous iron is oxidized to the ferric form for incorporation into ferritin. As the mucosal cells become saturated with ferritin, absorption ceases until the ferritin can be converted to transferritin for removal to the plasma. This involves reduction of the iron in ferritin to the ferrous form where it moves to the cell surface and is oxidized before incorporation into transferritin. As ferritin levels in the mucosal cells diminish, iron absorption increases. Therefore, there is an inverse relationship between mucosal ferritin levels and iron absorption. Iron deficient animals absorb dietary iron into the mucosal cells and convert the majority into transferritin while iron adequate animals convert only a small portion of the absorbed iron into transferritin for transport in the plasma (Conrad and Crosby, 1963). Callender et al. (1957) modified the mucosal theory to suggest that heme iron is directly absorbed into the mucosal cells with the porphyrin complex intact.

Factors Affecting Iron Absorption and Bioavailability

There are numerous factors which affect iron absorption and bioavailability such as age, iron status, species, dosage level, and other nutrient components of the diet both organic and inorganic. Furugouri and Kawabata (1976) using labeled ferric citrate showed that newborn pigs exhibited active absorption of iron up to 180 hours of age. Furugouri (1977) postulated that two mechanisms of active transport assist in iron absorption in the neonatal pig, namely endocytosis and absorption of ionic iron across the plasma membrane. Thoren-Tolling (1975) discovered that the neonatal pig is also able to absorb iron bound to macromolecules. Cornelius and Harmon (1973) reported that neonatal pigs are able to absorb considerable amounts of iron dextran via pinocytosis. This confirms previous work by Lecce et al. (1961), who proposed that large organic molecules such as iron dextran should be absorbed intact by pinocytosis. However, Miller et al. (1962) concluded that intestinal changes occur within 30 hours in nursing pigs preventing absorption of these intact macromolecules.

McCance and Widdowson (1937) proposed that efficiency of absorption was due primarily to the iron status of the animal. Bothwell et al. (1958) proposed that the two most important factors concerning iron absorption were iron stores and rate of erythropoiesis. This substantiates the mucosal block theory that an animal only absorbs what it needs or requires. Increasing levels of dietary iron lead to higher total amounts absorbed, however iron status of the animal is still more influential in determining iron absorption (Van Campen, 1974). Excess iron entering the mucosal cells of iron adequate

pigs is incorporated in ferritin, only to be lost later in the feces as a product of sloughed mucosal cells (Harmon et al., 1974).

Chausow and Czarnecki-Maulden (1988) noted species differences while completing a hemoglobin repletion study with beef liver and ferrous sulfate using cats and chicks. Iron in beef liver fed to cats was 350% as available as ferrous sulfate while only 90% as available in chicks. This, however, is not uncommon; if one looks at bioavailability tables there are several differences in bioavailability between species which is illustrated by the study of Pfau et al. (1977). That study showed the bioavailability of hemoglobin in pigs to be 50% relative to ferrous sulfate while Amine et al. (1972) derived a value of 70% using rats and chicks.

Work done with dosage level indicates higher absorption efficiencies with lower dosage levels. Pfau et al. (1977) demonstrated that the absorption efficiency of iron from either hemoglobin or ferrous sulfate to have an inverse relationship to dosage level. As stated previously, Van Campen (1974) reported that even though total amount absorbed may increase with higher doses, the efficiency of absorption decreases.

The physical or chemical form of iron also influences absorption. Iron from animal sources is more available than that from plant sources (Morris, 1987). This is due to the large proportion of heme iron in the animal sources. Heme iron is absorbed as an intact porphyrin complex whereas nonheme iron must be removed from its protein-bound complexes prior to absorption (Morris, 1987). Raffin et al. (1974) reported that once inside the mucosal cell, mucosal heme oxygenase cleaves the heme from the porphyrin ring releasing the iron into the same pathways as that of the nonheme fraction. Wheby and Spyker (1981) concluded this to be the rate limiting step in heme iron absorption from work with iron deficient dogs.

Various nutritive and nonnutritive elements within the diet have also been shown to affect iron absorption and thus, bioavailability. Waddell and Sell (1964) illustrated decreased iron absorption in chicks associated with increasing dietary concentrations of either calcium, phosphorus or both. Phosphorus has been hypothesized to affect iron absorption through the formation of insoluble ferric phosphate and phytate (Underwood, 1981). Bradley et al. (1983) illustrated that dietary Cu concentrations in the range of 120-240 parts per million. led to decreased liver Fe concentrations of 50-60% through possible impairment of iron absorption. Earlier, Gipp et al. (1974) concluded that Cu, when fed in diets at 250 parts per million., not only reduced iron absorption but could invoke iron-deficient anemia in pigs. Gubler et al. (1952) however noted that copper deficiency also hampered iron absorption. Another mineral that shows antagonistic effects on iron utilization is zinc when supplied in excess levels. Settlemire and Matrone (1967a, 1967b) concluded that zinc impacted iron via two methods; by impairment of iron incorporation into ferritin and by decreasing the life span of red blood cells leading to increased iron requirements. Dietary manganese in excessive levels leads to reductions in hemoglobin (Baker and Halpin, 1991).

While the aforementioned minerals have been shown to have detrimental effects on iron absorption and bioavailability, other nutritive factors have been shown to be beneficial. Three of these include the amino acids histidine, lysine and cysteine. Van Campen and Gross (1969) hypothesized that these amino acids form chelates with the ferric iron, thus keeping the iron in solution. These chelates have been identified as tridentate chelates. Vitamin C or ascorbic acid also has been shown to have beneficial effects upon iron absorption. Greenberg et al. (1957) concluded that iron-deficient rats had increased efficiency of absorption of iron when ascorbic acid was given with the iron supplement. This work supports previous findings of Moore and Dubach (1951) who reported increased food iron absorption in man through addition of ascorbic acid or foods containing ascorbic acid. Van Campen (1972) investigated the effects of histidine and ascorbic acid supplementation on iron absorption and concluded that ascorbic acid was more effective in increasing iron retention. This author hypothesized that this was due to the ability of ascorbic acid to act as both a reducing agent and a chelating agent. Rizk and Clydesdale (1983) meanwhile noted significant decreases in insoluble iron in soy protein isolate following addition of ascorbic acid.

Methods of Determining Bioavailability

Although several methods have been used to measure iron absorption and bioavailability, this review will be limited to hemoglobin repletion and use of radioisotopes. Hemoglobin as a source for quantifying iron bioavailability has been used since early in the 1920's (Mitchell and Schmidt, 1926). Two factors that make hemoglobin an attractive parameter to measure iron bioavailability are firstly the fact that hemoglobin accounts for roughly 60-80% of the body iron, thus making it a sensitive detector of differing absorption efficiencies, and secondly the relative ease in carrying out hemoglobin determinations. Within the hemoglobin repletion procedure, two different approaches have been used. Pla and Fritz (1970) used the methodology of supplementing an iron deficient diet with graded levels of an iron standard such as ferrous sulfate for creation of a standard curve. Bioavailability of test ingredients would then be determined by supplementation of the test ingredient to the basal diet. Relative bioavailability values were determined through the ratio of hemoglobin gain from the test source to that of the standard. The other common approach used in hemoglobin repletion studies was to compare the slope ratios of hemoglobin response from both the test and standard sources. Amine et al. (1972) developed this method in which graded levels of both the test sources and the standard were supplemented to a common iron deficient basal diet. Advantages of the hemoglobin repletion procedure are that numerous iron sources can be evaluated in a relatively short period of time and that it takes into account both absorption and utilization of the iron; however, the values derived are relative not absolute.

As stated by Smith (1983), methodologies utilizing radioactive tracers, especially extrinsic tagging, for the determination of nutrient bioavailability have become the most popular due to convenience. The overriding principle behind intrinsic tagging is that when a known quantity of radio-iron is added to the food or test ingredient complete isotope exchange occurs. Formation of a homogenous pool, labeled and nonlabeled, of nonheme iron is critical to the resulting bioavailabilities if validity is to be assured

(Smith, 1983). The radioactivity is then measured as the response parameter and expressed as a percentage of total iron content. However, in the presence of incomplete isotope exchange the values derived from radiotracers can be overestimates (Van Campen, 1983).

Deficiency Symptoms

Iron-deficient anemia, termed hypochromic-microcytic anemia is generally associated with young, rapidly growing animals deprived of iron in their diet or from their environment. The most common parameter to indicate Fe-deficient anemia is hemoglobin concentration. The National Research Council (1979) created a classification system in which pigs could be categorized to the extent of the anemia by their hemoglobin concentration, measured in grams/deciliter. Pigs exhibiting hemoglobin levels of ten or above are classified as normal, nine is the minimum level for optimum performance, eight indicates borderline anemia, seven is the level in which anemia retards growth rate, six is considered severe anemia and four as severe anemia with increased mortality. The first signs of iron-deficient anemia is often roughness of haircoat and loss of pigmentation or color of mucous membranes. The skin can become wrinkled and the pigs emit a general listlessness, characterized by drooping of the head and ears combined with a loss of appetite. Iron-deficient anemia was shown to reduce weight gains in affected pigs as early as the 1930's (Moe et al., 1935). In severe cases pigs may be characterized or identified by labored breathing, increased heart and respiratory rates, and even systolic murmurs due to reduced blood viscosity. The largest, fastest growing pigs are susceptible to sudden death from anoxia. Affected pigs have a higher prevalence for subcutaneous edema in the neck, shoulder and limb areas (Conrad et al., 1980). Osborne and Davis (1968) noted that anemic pigs showed higher susceptibility to infectious diseases. This coincides with later research by Nalder et al. (1972) which showed that dietary iron level was directly related to antibody production in weanling rats. Furthermore, Luke and Gordon (1950) showed evidence that anemic pigs were more susceptible to pneumonia, influenza and disorders of the alimentary tract.

Iron Requirements

The definition for net requirement of iron (Underwood, 1981) is the sum of the amounts laid down in the blood and tissues in the process of growth and the amounts lost in feces, urine, sweat, blood loss, at parturition, and in milk and eggs. The newborn pig contains approximately 50 milligram of iron at birth, mostly in the form of hemoglobin (Venn et al., 1947). The neonatal pig has been determined to have a requirement of 7 to 16 milligram of iron per day for normal growth (Venn et al, 1947). Another way in which this can be expressed is a need of 21 milligram of iron per kilogram of weight gain (Braude et al., 1962). Due to the minimal concentration of iron in sow's milk (1 milligram per liter) neonatal pigs reared in confinement require supplemental iron in order to overcome the susceptibility to anemia (Brady et al., 1978). Pigs not supplemented with iron while dependent on sows milk quickly develop iron-deficient anemia (Venn et al., 1947). Maximum hemoglobin levels were produced in neonate at 14 days of age when supplemented with either 100 or 150 milligram of iron dextran at birth

(Wahlstrom and Juhl, 1960). Maximum growth rate was acquired through supplementation of 100 milligram in the form of injectable iron dextran to pigs weaned at three weeks of age (Zimmerman et al., 1959). Iron requirement as a concentration of diet decreases with age and weight due to a decrease in blood volume per unit weight and higher iron intakes. The iron requirements for pigs 1 to 5 and 20 to 50 kilogram live weight are 100 and 60 parts per million respectively, which is equivalent to iron intakes of 25 and 114 milligram (National Research Council, 1988).

LITERATURE CITED

- Amine, E. K., R. Neff, and D. M. Hegsted. 1972. Biological estimation of available iron using chicks and rats. *J. Agr. Food Chem.* 20:247.
- Baker, D. H., and K. M. Halpin. 1991. Manganese and iron interrelationship in the chick. *Poult. Sci.* 70:146.
- Bothwell, T. H., G. Pirzio-Biroli, and C. A. Finch. 1958. Iron absorption. I. Factors influencing absorption. *J. Lab and Clin. Med.* 51:24.
- Boussingault, J.B. 1872. Du iron contenu dans le sang et dans les aliments. *Acad. Sci. Parts, Rend. Acad. Sci.* 74:1353.
- Braasch, 1891: (cited by Doyle et al., 1927).
- Bradley, B. D., G. Graber, R. J. Concon, and L. T. Frobish. 1983. Effects of graded levels of dietary copper on copper and iron concentrations in swine tissues. *J. Anim. Sci.* 56:625.
- Brady, P. S., P. K. Ku, D. E. Ullrey, and E. R. Miller. 1978. Evaluation of an amino acid-iron chelate haematinic for the baby pig. *J. Anim. Sci.* 47:1135.
- Braude, R., A. G. Chamberlain, M. Kotarbinska, and K. G. Mitchell. 1962. The metabolism of iron in piglets given labeled iron either orally or by injection. *Brit. J. Nutr.* 16:427.
- Bryan, C. P. 1931. *The papyrus ebers.* D. Appleton and Co., New York. p. 156.
- Bush, J. A., A. N. Jensen, G. E. Cartwright, and M. M. Wintrobe. 1955. Blood volume studies in normal and anemic swine. *Am. J. Physiol.* 181:9.
- Callender, S. T., B. J. Mallet, and M. D. Smith. 1957. Absorption of hemoglobin iron. *Brit. J. Haematol.* 3:186.
- Chausow, D. G., and G. L. Czarnecki-Maulden. 1988. The relative bioavailability of plant and animal sources of iron to the cat and chick. *Nutr. Res.* 8:1041.

- Cornelius, S. G., and B. G. Harmon. 1973. Absorption of oral iron dextran in neonatal pigs. *J. Anim. Sci.* 37:277 (Abstr.).
- Conrad, M. E., and M. H. Crosby. 1963. Intestinal mucosal mechanisms controlling iron absorption. *Blood.* 22:406.
- Conrad, H. R., D. R. Zimmerman, and G. F. J. Combs. 1980. NFIA, Literature Review on Iron in Animal and Poultry Nutrition. National Ironed Ingredients Association, West Des Moines, IA.
- Davies, H. G. 1961. Structure in nucleated erythrocytes. *J. Biophys. Biochem. Cytol.* 9:671.
- Doyle, L. P., F. P. Mathews, and R. A. Whiting. 1927. Anemia in young pigs. *Ind. J. Am. Vet. Med. Assoc.* 72:491.
- Elloit, J. I. 1976. Iron supplementation of piglet milk replacers. *Can. J. Anim. Sci.* 56:611.
- Fritz, J. C., G. W. Pla, T. Roberts, J. W. Boehne, and E. L. Hoover. 1970. Biological availability in animals of iron from common dietary sources. *J. Agric. Food Chem.* 18:647.
- Fruton, J. S., and S. Simmonds. 1958. *General Biochemistry*. 2nd Ed. John Wiley and Son, Inc., New York.
- Furugouri, K. 1977. Iron binding substances in the intestinal mucosa of neonatal piglets. *J. Nutr.* 107:487.
- Furugouri, K., and A. Kawabata. 1976. Iron absorption by neonatal pig intestine in vivo. *J. Anim. Sci.* 42:1460.
- Gipp, W. F., W. G. Pond, F. A. Kallironlz, J. B. Tasker, D. R. Van Campen, L. Krook, and W. J. Visek. 1974. Efiront of dietary copper, iron and ascorbic acid levels on hematology, blood and tissue copper, iron and zinc concentrations and ⁶⁴Cu and ⁵⁹Iron metabolism in young pigs. *J. Nutr.* 104:532.
- Greenberg, S. M., R. G. Tucker, A. E. Heming, and J. K. Mathues. 1957. Iron absorption and metabolism. I. Interrelationship os ascorbic acid and vitamin E. *J. Nutr.* 63:19.
- Gubler, C. J., M. E. Lahey, M. S. Chase, G. E. Cartwright, and M. M. Wintrobe. 1952. Studies on copper metabolism. III. The metabolism of iron in copper-deficient swine. *Blood.* 7:1075.

- Hahn, P. F., W. F. Bale, J. F. Ross, W. M. Balfour, and G. H. Whipple. 1943. Radioactive iron absorption by gastro-intestinal tract. *J. Exp Med.* 78:169.
- Harmon, B. G., S. G. Cornelius, J. Totsch, D. H. Baker, and A. H. Jensen. 1974. Oral iron dextran and iron from steel slats as hematinics for swine. *J. Anim. Sci.* 39:699.
- Hart, E. B., C. A. Elvehjem, H. Steenbock, G. Bohstedt, and J. M. Fargo. 1929. Anemia in suckling pigs. *Wisconsin Agr. Expt. Sta. Bull.* 409.
- Jensen, W. N., J. A. Bush, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe. 1956. The kinetics of iron metabolism in normal growing swine. *J. Exp. Biol. Med.* 103:145.
- Lecce, G., G. Matrone, and D. O. Morgan. 1961. Porcine neonatal nutrition: absorption of unaltered non-porcine proteins and polyvinyl-pyrrolidone from the gut of piglets and the subsequent effect on the maturation of the serum protein profile. *J. Nutr.* 73:158.
- Lee, G. R., R. S. Nacht, J. N. Lukens, and G. E. Cartwright. 1968. Iron metabolism in copper-deficient swine. *J. Clin. Invest.* 47:2058.
- Loosli, J. K. 1978. In *Proc. Latin American Symposium on Mineral Nutrition Research with Grazing Ruminants*.
- Luke, D., and W. A. W. Gordon. 1950. Observations on some pig diseases. *Vet. Rec.* 62:179.
- McCance, R. A., and E. M. Widdowson. 1937. Absorption and excretion of iron. *Lancet.* 233:680.
- McGowan, J. P. and A. Chrichton. 1924. Iron deficiency in pigs. *Biochem. J.* 18:265.
- Miller, E. R., B. G. Harmon, D. E. Ullrey, D. A. Schmidt, R. W. Luecke, and J. A. Hoeironr. 1962. Antibody absorption, retention and production by the baby pig. *J. Anim. Sci.* 21:309.
- Mitchell, H. S., and L. Schmidt. 1926. The relation of iron from various sources to nutritional anemia. *J. Biol. Chem.* 70:471.
- Moe, L. H., W. A. Craft, and C. P. Thompson. 1935. Supplementing soil with iron and copper for the prevention of anemia in young pigs. *J. Amer. Vet. Med. Assoc.* 87:302.
- Moore, C. V., and R. Dubach. 1951. Observations on the absorption of iron from foods tagged with radioiron. *Trans. Assoc. Amer. Physicians.* 64:245.

- Morris, E. R. 1987. In Trace Elements in Human and Animal Nutrition. (W. Mertz, ed) Academic Press, New York.
- Munro, H. N. 1977. Iron absorption and nutrition-introduction. *Iron. Proc.* 36:2015.
- Nalder, B. N., A. W. Mahoney, R. Romakrishnan, and D. G. Hendricks. 1972. Sensitivity of the immunological response to the nutritional status of rats. *J. Nutr.* 102:535.
- National Research Council. 1979. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Swine, Eighth Revised Ed. National Academy of Science-National Research Council, Washington, D. C.
- National Research Council. 1988. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Swine, Ninth Revised Ed. National Academy of Sciences, Washington, D. C.
- Osborne, J. C., and J. W. Davis. 1968. Increased susceptibility to bacterial endotoxin of pigs with iron-deficiency anemia. *J. Amer. Vet. Med. Assoc.* 152:1630.
- Pfau, A. K., Rudolphi, and H. C. Heinrick. 1977. Dosisabhängigkeit der intestinalen Eisenabsorption beim Saugironkel. *Zentbl. Vet Med., A.* 24:140.
- Pla, G. W., and J. C. Fritz. 1970. Availability of iron. *J. Assoc. Off. Anal. Chem.* 53:791.
- Raffin, S. B., C. H. Woo, K. T. Roost, D. C. Price, and R. Schmid. 1974. Intestinal absorption of hemoglobin iron-heme cleavage by mucosal heme oxygenase. *J. Clin. Invest.* 54:1344.
- Rizk, S. W., and F. M. Clydesdale. 1983. Efronct of iron sources and ascorbic acid on the chemical profile of iron in a soy protein isolate. *J. Food Sci.*
- Roeser, H. P., G. R. Lee, S. Nacht, and G. E. Cartwright. 1970. The role of ceruloplasmin in iron metabolism. *J. Clin. Invest.* 49:2408.
- SAS. 1990. SAS/STAT Users Guide (Version 6Ed.) SAS Institute Inc., Cary , NC.
- Settlemyre, C. T., and G. Matrone. 1967a. In vivo interironrence of zinc with ironrritin iron in the rat. *J. Nutr.* 92:153.
- Settlemyre, C. T., and G. Matrone. 1967b. In vivo efronct of zinc on iron turnover in rats and liiron span of the erythrocyte. *J. Nutr.* 92:159.
- Shoden, A., and P. Sturgeon. 1961. Formation of Haemosiderin and its Relation to Ironrritin. *Nature (London).* 189:846.

- Smith, K. T. 1983. Effects of chemical environment on iron bioavailability measurements. *Food Technol.* 37:115.
- Talbot, R. B., and M. J. Swenson. 1963. Survival of CR⁵¹ labeled erythrocytes in swine. *Proc. Soc. Exp. Biol. Med.* 112:573.
- Thoren-Tolling, K. 1975. Studies on the absorption of iron after oral administration in piglets. *Acta Vet. Scand. Suppl.* 54.
- Ullrey, D. E., E. R. Miller, O. A. Thompson, I. M. Ackermann, D. A. Schmidt, J. A. Hoeironr, and R. W. Luecke. 1960. The requirement of the baby pig for orally administered iron. *J. Nutr.* 70:187.
- Underwood, E. J. 1981. *The Mineral Nutrition of Livestock.* Commonwealth Agricultural Bureaux, London, England.
- Van Campen, D. 1972. Effect of histidine and ascorbic acid on the absorption and retention of ⁵⁹Iron by iron-depleted rats. *J. Nutr.* 102:165.
- Van Campen, D. 1974. Regulation of iron absorption. *Iron. Proc. Iron. Am. Soc. Exp. Biol.* 33:100.
- Van Campen, D. 1983. Iron bioavailability techniques: an overview. *Food Technol.* 37:127.
- Van Campen, D., and E. Gross. 1969. Effect of histidine and certain other amino acids on the absorption of iron-59 by rats. *J. Nutr.* 99:68.
- Venn, J. A. J., R. A. McCance, and E. M. Widdowson. 1947. Iron metabolism in piglet anemia. *J. Comp. Pathol. Therap.* 57:314
- Waddell, D. G., and J. L. Sell. 1964. Effects of dietary calcium and phosphorus on the utilization of dietary iron by the chick. *Poult. Sci.* 43:1249
- Wahlstrom, R. C. and E. W. Juhl. 1960. A comparison of different methods of iron administration on rate of gain and hemoglobin level of the baby pig. *J. Anim. Sci.* 19:183.
- Wheby, M. S., and D. A. Spyker. 1981. Hemoglobin iron absorption kinetics in the iron-deficient dog. *Am. J. Clin. Nutr.* 34:1686.
- Withrow, G., and M. C. Bell. 1969. Erythrocytic Iron Span Estimations in Growing Sheep and Swine Using ⁷⁵Se. *J. Anim. Sci.* 28:240.

Zimmerman, D. R. 1980. Iron in swine nutrition. In National Ironed Ingredient Association Literature Review on Iron in Animal and Poultry Nutrition. West Des Moines, IA: National Ironed Ingredient Association.

Zimmerman, D.R., V. C. Speer, V. W. Hays, and D. V. Catron. 1959. Injectable iron dextran and several oral iron treatments for the prevention of iron-deficiency anemia of baby pigs. *J. Anim. Sci.* 18:1409.