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EFFECTS OF IRON SUPPLEMENTATION ON BINDING ACTIVITY OF IRON REGULATORY PROTEINS AND THE SUBSEQUENT IMPACT ON GROWTH PERFORMANCE AND INDICES OF HEMATOLOGICAL AND MINERAL STATUS

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EFFECTS OF IRON SUPPLEMENTATION ON BINDING ACTIVITY OF IRON REGULATORY PROTEINS AND THE SUBSEQUENT IMPACT ON GROWTH PERFORMANCE AND INDICES OF HEMATOLOGICAL AND MINERAL STATUS OF YOUNG PIGS

By

Michael James Rincker

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ABSTRACT

EFFECTS OF IRON SUPPLEMENTATION ON BINDING ACTIVITY OF IRON REGULATORY PROTEINS AND THE SUBSEQUENT IMPACT ON GROWTH PERFORMANCE AND INDICES OF HEMATOLOGICAL AND MINERAL STATUS OF YOUNG PIGS

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Two experiments were conducted to evaluate the effects of supplemental Fe on binding activity of iron regulatory proteins (IRPs) and the subsequent impact on growth performance and indices of hematological and mineral status of young pigs. In Exp. 1, littermate male pigs (n = 10; 1.8 kg; 14 ± 1 h) were allotted by BW to treatments (5 pigs/treatment). Treatments were administered by i.m. injection: 1) Sal (1 mL of sterile saline solution); 2) Fe (1 mL of 200 mg Fe as Fe-dextran). Pigs were bled (d 0 and 13) to determine hemoglobin (Hb), hematocrit (Hct), transferrin (Tf), and plasma Fe (PFe) and then killed (d 13) to determine spontaneous and 2-mercaptoethanol (2-ME) inducible IRP binding activity in liver, and, liver and whole-body mineral concentrations. Pigs (n = 5; 2.2 kg; 14 ± 2 h) were also killed at d 0 to establish baseline (BL1) measurements for IRP binding activity and liver and whole-body mineral concentrations. In Exp. 2, pigs (n = 225; 6.5 kg; 19 ± 3 d) were randomly allotted by BW, litter, and gender to treatments (5 pigs/pen; 9 pens/treatment). Basal diets for each phase (Phase 1: d 0 to 7; Phase 2: d 7 to 21; Phase 3: d 21 to 35) were formulated to contain minimal Fe concentration and then supplemented with 0, 25, 50, 100, or 150 mg Fe/kg of diet as ferrous sulfate. Three pigs per pen (n = 135) were chosen and bled periodically (d 0, 7, 21, and 35) to determine Hb, Hct, Tf, and PFe. Whole-body and liver mineral concentrations were determined in five pigs (BL2; n = 5; 5.9 kg; 19 ± 3 d) killed at d 0 and 30 pigs (6 pigs/treatment) killed at d

35. Liver samples from BL2 pigs and pigs fed 0 or 150 mg of added Fe/kg of diet were analyzed for IRP binding activity. In Exp. 1, no difference ($P = 0.482$) was observed in ADG. On d 13, Fe treated pigs had greater ($P = 0.001$) Hb, Hct, and PFe and lower ($P = 0.002$) Tf than Sal treated pigs. Whole-body ($P = 0.002$) and liver Fe concentration were greater ($P = 0.001$) in Fe vs. Sal treated pigs, while the liver Fe concentration of Sal treated pigs was less ($P = 0.004$) than BL pigs. Sal treated pigs had greater ($P = 0.004$) spontaneous IRP binding activity compared with Fe treated pigs. In Exp. 2, the improvements in growth performance during Phase 2 (ADG, linear, $P = 0.036$; ADFI, linear, $P = 0.096$; G:F, quadratic, $P = 0.075$) were of sufficient magnitude that dietary treatments tended to increase overall ADG (linear, $P = 0.084$), ADFI (quadratic, $P = 0.095$), and G:F (quadratic, $P = 0.107$). Dietary Fe supplementation resulted in a linear increase in Hb, Hct, and PFe on d 21($P < 0.050$) and 35 ($P = 0.001$). Dietary treatments decreased (linear, $P = 0.004$) Tf on d 35. Whole-body and liver Fe concentrations increased (linear, $P < 0.010$) in pigs due to dietary treatments. The liver Fe concentration of all pigs killed on d 35 was less ($P = 0.001$) than BL2 pigs. Spontaneous ($P = 0.013$) and 2-ME inducible ($P = 0.005$) IRP binding activities were greater in pigs fed diets containing 0 vs. 150 mg of added Fe/kg of diet. Results indicate that IRP binding activity is influenced by Fe supplementation. Subsequently, other indicators of Fe status are affected via IRP's role in post-transcriptional expression of Fe storage and transport proteins. The decrease in Fe stores was not severe enough to reduce growth performance. Even so, the lessening of a pig's Fe stores during this rapid growth period may result in the occurrence of anemia during later growth stages.

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LIST OF ABBREVIATIONS

A: Adenine

BL: Baseline Pigs

C: Cytosine

c-Acon: cytosolic-Aconitase

Dcyt*b*: Duodenal cytochrome *b*

DCT1: Divalent Cation Transporter 1

eALAS: erythroid-Aminolevulinate Synthase

Fe^{+2}: Ferrous Iron

Fe^{+3}: Ferric Iron

$FeSO_4$: Ferrous Sulfate

Fr: Ferrireductase

H-Ferritin: Heavy-Ferritin

Hb: Hemoglobin

Hct: Hematocrit

HFE: Human Factors Engineering

Hm: Heme

HO: Heme Oxygenase

Hp: Hephaestin

G: Guanine

Ig: Integrin

IRE: Iron Response Element

Ireg1: Iron-regulated transporter 1

IRP: Iron Regulatory Protein

L-Ferritin: Light-Ferritin

M: Methyl

m-Acon: mitochondrial-Aconitase

Mf: Mobilferrin

mRNA: Messenger RNA

MTP1: Metal Transporter Protein 1

Nramp2: Natural resistance-associated macrophage protein 2

PA: Propionic Acid

PCu: Plasma Copper

Pf: Paraferritin

PFe: Plasma Iron

PZn: Plasma Zinc

RBV: Relative Biological Value

SDH: Succinate Dehydrogenase

TCA: Tricarboxylic Acid

Tf: Transferrin

TfR: Transferrin Receptor

TIBC: Total Iron-Binding Capacity

U: Uracil

TIBC: Unsaturated Iron-Binding Capacity

UTR: Untranslated Region

V: Vinyl

X: Any Nucleotide

2-ME: 2-Mercaptoethanol

INTRODUCTION

Several factors contribute to the potential development of Fe deficiency in young pigs. The hepatic Fe stores of a newborn pig combined with the low Fe concentration in sow's milk are not sufficient to meet the rapid growth and increase in blood volume. Consequently, the use of an exogenous source of Fe to prevent Fe deficiency in young pigs has been well documented (Ullrey et al., 1959; Kernkamp et al., 1962; Pollmann et al., 1983) and is standard practice in the swine industry.

Similar to the suckling phase, the nursery phase is also characterized by increased blood volume and rapid growth. The NRC (1998) postweaning dietary Fe requirement, 80 mg/kg, is based on early experiments conducted by Pickett et al. (1960) who reported decreased growth in pigs weaned at 10 to 14 d of age and fed diets containing 60 mg added Fe/kg diet, and decreased hemoglobin and hematocrit in pigs fed diets containing 80 mg added Fe/kg diet. Thus, there is a need to reevaluate the dietary Fe requirement using genetics and management practices representative of the swine industry today.

Iron regulatory proteins (IRPs) are regarded as critical determinants of Fe homeostasis because they modulate the post-transcriptional expression of proteins required for the transport, storage, and use of Fe (Eisenstein, 2000). Located within the cytoplasm of cells, IRPs bind to a highly conserved, 28-bp nucleotide sequence known as an iron responsive element (IRE) sequence (Gray et al., 1996). Examples of proteins whose transcribed messenger-RNA contain an IRE include erythroid aminolevulinate synthase, transferrin receptor, and H- and L-ferritin (Eisenstein, 2000). The binding activity of IRPs is influenced by Fe status. Iron deficiency causes an increase in binding activity while Fe sufficiency decreases binding activity (Zahringer et al., 1976; Cox and

Adrian, 1993). Researchers have shown in the rat that IRP binding activity is responsive to dietary Fe (Chen et al., 1997; Chen et al., 1998). However, IRP binding activity has not been investigated in the pig. By determining IRP binding activity in addition to the traditional measurements (i.e. Hb concentration and Hct percentage) used to establish Fe status, a better assessment of a young pig's Fe state can be made.

The research objectives were to determine the effects of Fe supplementation on binding activity of iron regulatory proteins and the subsequent impact on growth performance and indices of hematological and mineral status of young pigs. Experiments compared neonatal pigs administered Fe-dextran vs. saline, as well as, evaluated increasing concentrations of supplemental dietary Fe as ferrous sulfate in nursery pigs.

CHAPTER ONE

LITERATURE REVIEW

History

Amongst all the microminerals, the history of Fe is the longest and best described. As early as 1500 BC, the Egyptian civilization was known to have used Fe compounds for health and disease purposes, followed by the Hindus, Greeks, and Romans somewhat later (Vannotti and Delachaux, 1949). The first description of anemia surfaced in published accounts in 1554, with Johannes Lange's description of "the disease of virgins" (Major, 1932). Later in 1615, the Greek physician Jean Varandal gave the name chlorosis, which means green, to the symptoms of anemia (Mettler, 1947). Although the relationship between Fe and blood was shown in the sixteenth century, the physiological basis was not discovered until 1886 when Zinoffsky revealed that horse hemoglobin (Hb) contained 0.34% Fe (Underwood and Suttle, 1999). The first reported investigation relating to dietary Fe consumption was provided by Stockman (1895) who determined that patients suffering from chlorosis consumed 1.3 to 3.0 mg of Fe/d as compared with 6.0 to 11.0 mg of Fe/d consumed by healthy subjects. As well, Heath et al. (1932) provided convincing evidence that inorganic Fe supplements could be used for Hb synthesis when they reported that the amount of parenteral Fe administered was highly correlated to the increased quantity of Fe in circulating Hb. The first published evidence associating Fe deficiency with baby pig anemia was in 1924 (McGowan and Crichton).

Chemical Properties

The inorganic element Fe is the second most abundant metal and the fourth greatest element, comprising approximately 4.7% of the earth's crust (Beard and Dawson, 1997).

Iron is a silvery-white or gray color and somewhat magnetic. As element 26 within the periodic table, Fe has an atomic weight of 55.85 and is located in the middle of the first transition series of elements. Due to its location, Fe has the possibility of multiple oxidation states ranging from Fe^{-2} to Fe^{+6}. Even so, the primary oxidation states are the ferrous (Fe^{+2}) and ferric (Fe^{+3}) ions, which are stable in aqueous solutions. Iron complexes are important in electron transfer reactions at the cellular level because of the ease at which Fe changes between these two oxidation states. However, this capability also results in Fe being potentially toxic, as it is involved in the Fenton reaction that chemically converts superoxide to extremely reactive hydroxyl radicals, which can damage cells (Fenton, 1894).

Dietary Sources and Availability

Various sources of Fe may be supplemented in the diet to meet the Fe requirement of animals and prevent the occurrence of Fe deficiency. Ferrous sulfate ($FeSO_4$) is most commonly used by the feed industry as a dietary Fe source because it is essentially 100% bioavailable (Baker, 2001). Therefore, Fe bioavailability of other sources is determined relative to $FeSO_4$. The increase in whole blood Hb concentration of Fe-depleted animals is the method commonly used to determine the Fe availability from various sources. Fritz et al. (1970) calculated the relative biological value (RBV) of various Fe sources with the following formula using $FeSO_4$ as a standard: RBV, % = [(mg of Fe/kg of diet from $FeSO_4 \cdot 7H_2O$ for measured Hb response ÷ mg of Fe/kg of diet from Fe source for an equal Hb response) X 100]. The high RBV for ferric chloride (98%), ferric citrate (100%), ferric choline citrate (102%), and ferric ammonium citrate (100%) make them good sources of dietary Fe (Fritz et al., 1970). The availability of Fe in ferrous carbonate

is lower than $FeSO_4$ (Ammerman et al., 1974), while ferric oxide is almost completely unavailable and ineffective in meeting an animal's Fe requirement (Ammerman and Miller, 1972).

Many feed ingredients commonly included in swine diets provide some form of Fe. However, the Fe concentration of these feed ingredients is highly variable and dependent upon such factors as soil type, environmental conditions, or processing procedures. This is especially true of the Fe concentration found in plant materials used as feedstuffs. The Fe concentration in cultivated grasses and legumes is known to range from 100 to 700 mg of Fe/kg, although values in excess of 1,000 mg of Fe/kg have also been noted (Beeson, 1941). Mitchell (1963) reported that the Fe concentration in soils was 20 to 100 times greater than that of the forage grown on that soil. A high Fe concentration is most likely to occur in soils subject to heavy periods of waterlog (Underwood and Suttle, 1999). Hematite and maghemite are two common Fe oxides that are prevalent in highly weathered soils. It is hematite that gives many red soils their color (Sparks, 2003).

The Fe concentration of most cereal grains ranges from 30 to 60 mg of Fe/kg (NRC, 1998). However, the Fe in cereal grains is largely complexed with phytate or fiber, forming insoluble chelates and lowering its bioavailability (Baker, 2001). Chausow and Czarnecki-Maulden (1988a) reported that the bioavailability of Fe in yellow corn was 20%. In comparison to cereal grains, leguminous seeds and oilseed meals typically have a greater concentration of Fe, ranging from 100 to 200 mg of Fe/kg, but again the bioavailability is lowered because of insoluble complexes with phytate or fiber (Baker, 2001). Chausow and Czarnecki-Maulden (1988a; 1988b) reported the Fe bioavailability of dehulled soybean meal, sesame meal and alfalfa meal to be 45, 96, and 65%,

respectively. A similar value (38.5%) for the Fe bioavailability of soybean meal was reported by Biehl et al. (1997). These authors also noted that supplementation of either phytase or 1-α-hydroxycholecalciferol did not improve the bioavailability of Fe in soybean meal (Biehl et al., 1997). However, Stahl et al. (1999) reported that dietary phytase effectively degraded phytate in corn-soybean meal diets and subsequently released phytate bound Fe, improving the bioavailability of dietary Fe.

Feedstuffs of animal origin, other than milk and milk products, are good sources of dietary Fe. The Fe concentration in meat meal, fish meal, and poultry by-product meal commonly ranges from 400 to 600 mg of Fe/kg, while blood meal generally contains 2,000 to 3,000 mg of Fe/kg (NRC, 1998). Chausow and Czarnecki-Maulden (1988a; 1988b) reported the Fe bioavailability relative to $FeSO_4$ to be 48, 32, 68, and 22% for meat and bone meal, fish meal, poultry by-product meal, and dried blood meal, respectively. However, Miller (1978) reported that the bioavailability of Fe in dried blood meal ranged from 40 to 50%. These differences suggest that the processing techniques can greatly influence the bioavailability of Fe in feedstuffs originating from animal sources. Recent technological advancements in processing techniques, including spray-drying and flash-drying procedures, have improved the consistency of animal protein sources and helped to provide more accurate estimates of Fe bioavailability.

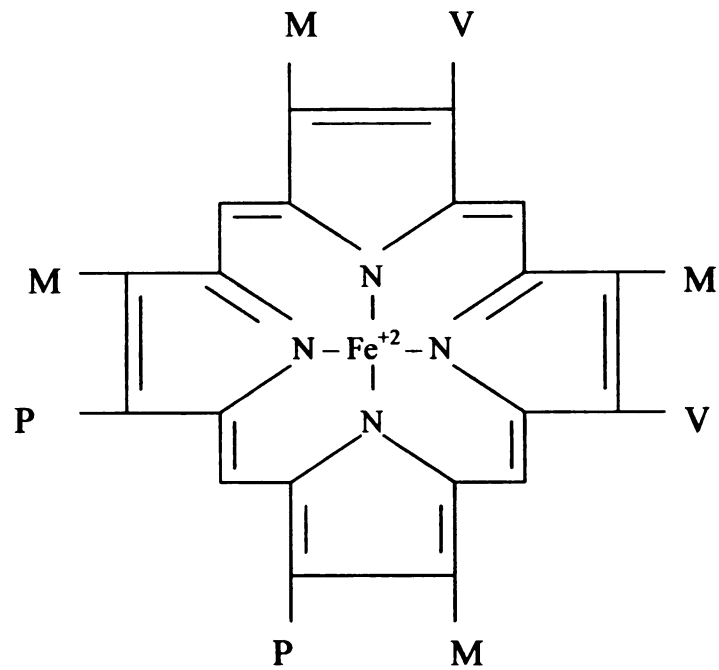
Tissue Distribution and Function

Iron is an essential nutrient for all living organisms, excluding a select few members of the bacterial genera *Lactobacillus* and *Bacillus* (Beard and Dawson, 1997). Bothwell et al. (1958) estimated that a 70 kg adult human has a whole body Fe concentration of 60 to 70 mg of Fe/kg. The Fe concentration in the pig at birth is approximately 20 to 30 mg

of Fe/kg (Venn et al., 1947); of which, 47% is associated with blood, 15% is in the liver, 1.6% is in the spleen, and the remaining 44% is found in other body tissues (Thoren-Tolling, 1975). Within the body, Fe is bound to proteins forming complexes that are classified as either heme or nonheme complexes. The primary heme proteins are Hb in red blood cells, myoglobin in muscle, and cytochromes throughout the body. Nonheme complexes include transferrin (Tf) in serum, uteroferrin in placenta, lactoferrin in milk, and ferritin and hemosiderin in the liver. These Fe-protein complexes are key intermediates in the transport, storage, and utilization of Fe by the body (Beard and Dawson, 1997).

Synthesis of heme groups occurs primarily in erythrocytes and the liver and accounts for the largest utilization of Fe by the body. Heme is an Fe-containing prosthetic group that contains a centrally located Fe^{+2} ion within four pyrrole rings (Voet et al., 1999). The structure of heme is presented in Figure 1. Hemoglobin and the catalases contain four heme groups per molecule, whereas myoglobin, cythochromes, and peroxidases contain one heme group per molecule.

Figure 1. Structure of heme[ab]



[a]Letter abbreviations indicate side chains: PA (propionic acid), —CH_2—CH_2—COO^-; V (vinyl) —$CH{=}CH_2$; M (methyl) —CH_3.
[b]Adapted from Mathews et al. (2000).

Hemoglobin. Hemoglobin represents greater than 95% of the protein in erythrocytes (Brody, 1999) and accounts for 60 to 80% of total body Fe (Miller et al., 1981). Hemoglobin is a tetrameric protein with two pairs of identical polypeptide chains (Beard and Dawson, 1997). Each polypeptide chain contains a heme group where the Fe^{+2} ion reversibly binds oxygen. The primary function of Hb is to bind oxygen in the lungs and deliver it to tissues for use. A remarkable feature of Hb is its ability to become fully oxygenated during the short erythrocyte transit time in pulmonary circulation (Yip and Dallman, 1996). Following the release of oxygen, Hb plays a second role by removing carbon dioxide (a major product from metabolite oxidation) in venous blood to the lungs for exhalation. The average life span of erythrocytes is 70 d in swine (Withrow and Bell, 1969) and 120 d in humans (Beard, 2001).

Myoglobin. Muscle tissue requires a large oxygen reserve for periods when energy demands are high. Myoglobin functions in the muscle both as an oxygen storage protein and to facilitate the transport of oxygen for use in energy production. Similar to Hb, myoglobin is a globular protein consisting of a heme group which is surrounded by a long protein known as a globin moiety (Clydesdale and Francis, 1971). Prior to slaughter of an animal, myoglobin accounts for 10% of the total body Fe (Clydesdale and Francis, 1971). Three principal forms of myoglobin exist, these being reduced myoglobin, oxymyoglobin, and metmyoglobin. Fresh meat color is determined by the relative proportions and distributions of these three forms of myoglobin (Watts et al., 1966). For example, oxymyoglobin is the most desirable color for fresh meats and gives meat its bright color, while metmyoglobin is associated with brown pigment.

Cytochromes, Peroxidases and Catalases. Another key group of heme containing proteins includes peroxidases, catalases, the cytochrome P_{450} family, and cytochrome oxidase. Catalases (EC 1.11.1.6) and peroxidases (EC 1.11.1.7) both function to metabolize peroxide molecules via the heme Fe located in the enzyme's active site (Mathews et al., 2000). The cytochrome P_{450} family has several hundred enzyme activities attributed to it including participation in the biosynthesis of steroid hormones and formation of unsaturated fatty acids (Beard and Dawson, 1997). Cytochrome oxidase (EC 1.9.3.1) is the terminal enzyme of the mitochondrial electron transport chain and catalyzes the reduction of molecular dioxygen to water which is required for ATP synthesis (Crichton, 2001). Keilin (1925) reported the characteristic absorption bands of the three cytochromes *a*, *b*, and *c* and determined that they were involved in the mitochondrial electron transport chain. The cytochromes function as electron carriers by cycling Fe between the Fe^{+2} and Fe^{+3} states, linking the oxidation of a substrate with the reduction of molecular oxygen in aerobic metabolism (Voet et al., 1999).

Ferritin. Ferritin is the primary nonheme storage protein of Fe and is found predominantly in hepatocytes, reticuloendothelial cells, and bone marrow (Beard and Dawson, 1997). The overall structure of apoferritin is conserved among higher eukaryotes including humans (Beard and Dawson, 1997), pigs (Collawn et al., 1987), and rats (Chen et al., 1998). Apoferritin is composed of 24 polypeptide subunits which join together to form a structure that has a hollow protein shell (Crichton and Ward, 1992). The core of the apoferritin shell can bind up to 4,500 Fe^{+3} ions (Fischbach and Anderegg, 1965); however, approximately 20% (800 of 4,500) of the ferritin shell is typically saturated with Fe^{+3} ions (Cook and Skikne, 1982). Two distinct isoforms (H – heavy; L –

light) of the polypeptide subunit exist. H-Ferritin has a molecular weight of 22-kDa and contains 182 amino acids while L-ferritin has a molecular weight of 20-kDa and is composed of 174 amino acids (Beard and Dawson, 1997). The ferritin isoforms of the horse, rat, and human are somewhat tissue specific; H-ferritin predominates in the heart and brain, while L-ferritin is found primarily in the liver and spleen (Arosio et al., 1978). However, the distribution of ferritin isoforms deviates in the pig with the liver containing approximately 60% H-ferritin and 40% L-ferritin and the spleen being comprised of 50% of each ferritin isoform (Collawn and Fish, 1984). Another difference between the H- and L-isoforms is that H-ferritin is a ferroxidase that facilitates rapid incorporation of Fe into ferritin shells, whereas Fe binds more slowly but stays attached longer to L-ferritin (Eisenstein, 2000).

Hemosiderin. Hemosiderin is another form of nonheme Fe storage that is present in small amounts in normal tissues but increases in abundance during periods of Fe overload (Crichton and Ward, 1992). Hemosiderin is a water insoluble protein that is derived from ferritin after lysosomal degradation of its protein shell (Weir et al., 1984). Hemosiderin has the capability to store different forms of Fe including amorphous ferric oxide, ferrihydrite, and goethite (Crichton and Ward, 1992). These forms of Fe are less chemically reactive compared with forms stored in ferritin and may be less available for mobilization from the cell (Beard and Dawson, 1997).

Transferrin. The Tfs are a family of proteins which includes serum Tf, ovotransferrin, lactoferrin, melanotransferrin, and hemiferrin (Beard and Dawson, 1997). Serum Tf is the primary transport protein that is responsible for distributing Fe within the body. The main routes of Fe transport are from intestinal absorption sites and storage

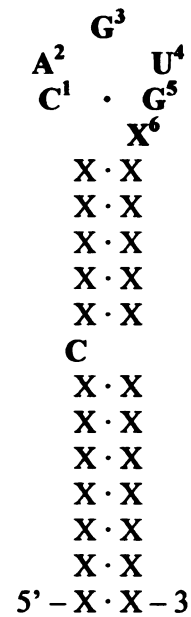
sites to reticulocytes for hematopoiesis. Less than 1% of the total body Fe is in the transport pool (Yip and Dallman, 1996). Transferrin is synthesized primarily in the liver and both Idzerda et al. (1986) and McKnight et al. (1980) have reported that transcription of Tf mRNA is inversely correlated to bodily Fe status. Serum Tf is an 80-kDa single chain protein that has the capacity to bind two Fe^{+3} ions (Beard and Dawson, 1997). Ceruloplasmin (EC 1.16.3.1), a Cu-containing plasma glycoprotein, is required for the incorporation of Fe into apotransferrin in the Fe^{+3} form by its ferroxidase activity (Lindley, 1996). Typically, one-third of the Tf binding sites in plasma are saturated with Fe^{+3} ions (Bothwell et al., 1979). As a means of preventing the accumulation of unbound Fe, which can have prooxidant effects, excess quantities of apotransferrin are present under normal physiological conditions (Beard and Dawson, 1997).

Iron Regulatory Proteins

Iron regulatory proteins (IRPs) are regarded as critical determinants of cellular Fe homeostasis because of the posttranscriptional regulation they exhibit on expression of proteins required for the uptake, storage, and utilization of Fe. Located within the cytoplasm of cells, IRPs bind to a highly conserved, 28-basepair nucleotide sequence known as an iron response element (IRE) sequence (Gray et al., 1996). An IRE sequence is situated within either the 5'- or 3'-untranslated region (UTR) of messenger-RNA (mRNA) and is comprised of the purines, adenine (A) and guanine (G), and the pyrimidines, cytosine (C) and uracil (U). The overall structure of an IRE sequence is a stem loop configuration composed of the conserved loop sequence, CAGUGX where X is usually U or C but can be an A (Eisenstein and Blemings, 1998). A key factor in the recognition of an IRE by an IRP is a bulged nucleotide region in the mRNA stem that is

located five nucleotides before the first nucleotide of the loop (Bettany et al., 1992). These authors also reported that the binding affinity between an IRP and an IRE was reduced by either disrupting the base pairing in the upper stem, by extending the size of the loop, or by extending the distance between the loop and the bulged nucleotide region (Bettany et al., 1992). The consensus sequence of an IRE is presented in Figure 2.

Figure 2. Consensus iron response element with single cytosine bulge[ab]



[a]Adapted from Hentze and Kuhn (1996).
[b]Letter abbreviations indicate nucleotides: A (adenine); G (guanine); C (cytosine); U (uracil); X (any nucleotide)

Examples of proteins whose transcribed mRNA contain an IRE include H- and L-ferritin (Aziz and Munro, 1987) and transferrin receptor (TfR) (Casey et al., 1988). This highly conserved IRE sequence exhibits almost perfect homology in both H- and L-ferritin mRNAs of the human (Costanzo et al., 1986; Santoro et al., 1986), rat (Leibold and Munro, 1987; Murray et al., 1987), chicken (Stevens et al., 1987), and frog (Didsbury et al., 1986). By having posttranscriptional control over proteins involved in Fe transport and storage, IRPs are key regulators of Fe homeostasis.

May et al. (1990) reported that erythroid-aminolevulinate synthase (eALAS; EC 2.3.1.37), the rate controlling enzyme in the heme biosynthesis pathway, contains an IRE in the 5'-UTR of its mRNA. This would suggest that IRPs link the synthesis of protoporphyrin IX with the availability of Fe in order to coordinate these two facets of heme synthesis.

Dandekar et al. (1991) reported that mitochondrial aconitase (m-Acon) also contains an IRE in the 5'-UTR of its mRNA. Mitochondrial aconitase (EC 4.2.1.3) functions in the tricarboxylic acid (TCA) cycle by converting citrate to isocitrate (Voet et al., 1999). Another TCA cycle enzyme in *Drosophila melanogaster*, succinate dehydrogenase (SDH) Fe protein subunit, has been found to contain an IRE sequence (Kohler et al., 1995). Succinate dehydrogenase (EC 1.3.99.1) catalyzes the dehydrogenation of two saturated carbons to a double bond, converting succinate to fumarate (Mathews et al., 2000).

Recently, researchers have reported that the mRNA for a metal ion transporter protein bound to the cellular membrane contains an IRE sequence. Natural resistance-associated macrophage protein 2 (Nramp2), also known as divalent cation transporter 1 (DCT1),

functions as a brush border Fe transport protein (Gruenheid et al., 1995). Transcription of DCT1 results in two alternatively spliced mRNAs, one of which contains an IRE in its 3'-UTR. A deficiency in dietary Fe results in increased translation of the mRNA isoform containing the IRE (Gunshin et al., 1997; Canonne-Hergaux et al., 1999). Three independent groups have identified a duodenal Fe-export protein located at the basolateral membrane of the enterocyte. It was named iron-regulated transporter 1 (Ireg1) by McKie et al. (2001), ferroportin by Donovan et al. (2000), and metal transporter protein 1 (MTP1) by Abboud and Haile (2000). McKie et al. (2001) reported that Ireg1 contains a functional IRE in the 5'-UTR of the mRNA and that both mRNA and protein expression are upregulated in response to increased Fe absorption. Abboud and Haile (2000) reported that MTP1 is expressed in tissues involved in Fe homeostasis, including the reticuloendothelial system, the duodenum, and the uterus of pregnant mice. The presence of transporter proteins on the cellular membrane suggests a means by which IRPs regulate the cellular Fe import or export based upon its need. The IRE sequence in the mRNA for various proteins is listed in Figure 3.

Figure 3. Alignment of untranslated regions containing an iron response element sequence[abc]

Consensus:	•••••• C ••••• CAGUGX ••••••••••
Porcine m-acon:	CCUCAUCUUUGUCAGUGCACAAAAUGGCG
Bovine m-acon:	CCUCAUCUUUGUCAGUGCACAAAAUGGCG
Human m-acon:	CCUCAUCUGUCAGUGCACAAAAUGGCGC
Human H-ferritin:	UUCCUGCUUCAACAGUGCUUGGACGGA
Human L-ferritin:	CUCUUGCUUCAACAGUGUUUGACGAACA
Rat L-ferritin:	AUCUUGCUUCAACAGUGUUUGGACGGAAC
Human eALAS:	UUCGUUCGUCCUCAGUGCAGGGCAACAGG
Murine eALAS:	UGGUUGCGUCCUCAGUGCAGGGCAACAG
Drosophila SDH:	UAAUUGCAAACGCAGUGCCGUUUCAAUUG
Human TfR A:	AUUUAUCAGUGACAGAGUUCACUAUAAAU
Human TfR B:	AAUUAUCGGAAGCAGUGCCUUCCAUAAUUAU
Human TfR C:	CAUUAUCGGGAGCAGUAUCUUCCAUAAUGU
Human TfR D:	UAUCGGAGACAGUGAUCUCCAUA
Human TfR E:	AAUUAUCGGGAACAGUGUUUCCCAUAAUU
Human MTP1:	AACUUCAGCUACAGUGUUAGCUAAGUUU
Murine MTP1:	AACUUCAGCUACAGUGUUAGCUAAGUUU

[a]Abbreviations: m-acon (mitochondrial aconitase); eALAS (erythroid δ-aminolevulinate synthase); SDH (succinate dehydrogenase); TfR (transferrin receptor); MTP1 (metal transporter protein 1).
[b]Underlined nucleotides signify the bulged-nucleotide region in the mRNA stem and the conserved loop sequence.
[c]Adapted from Aziz and Munro (1987), Kohler et al. (1995), Guggenheim (1995), Gray et al. (1996), Kim et al. (1996), Beard and Dawson (1997), and Abboud and Haile (2000).

The IRE sequence in H- and L-ferritin, eALAS, m-acon, and SDH Fe protein subunit is located in the 5'-UTR of mRNA, whereas the IRE sequence in TfR and DCT1 is found in the 3'-UTR of mRNA. The location of the IRE determines whether binding activity inhibits or stabilizes translation of the mRNA. The binding of an IRP to an IRE in the 5'-UTR inhibits the ribosome from initiating translation of the mRNA by preventing the cap binding complex, eukaryotic-initiation factor-4F, from making functional contact with the 43S preinitiation complex of the ribosome (Muckenthaler et al., 1998). Whereas the binding of an IRP to an IRE in the 3'-UTR stabilizes the mRNA and prevents degradation (Guo et al., 1995b).

Iron status determines the binding activity of IRPs. When Fe concentrations are low, IRPs are high affinity ($K_d \sim 20$ to 100 pmol/L) mRNA binding proteins (Eisenstein and Blemings, 1998). An excess of Fe results in IRPs being low affinity RNA binding proteins, causing their dissociation from the targeted mRNA (Chen et al., 1998).

Two isoforms of IRP have been identified, IRP1 and IRP2, with both forms exhibiting similar affinity for an IRE sequence (Chen et al., 1998). Iron regulatory protein 1 is approximately 90-kDa and is identical to cytosolic aconitase (c-acon) in amino acid composition, molecular weight, and isoelectric point (Kennedy et al., 1992). Because of these similarities, both IRP1 and c-acon can function as mRNA binding proteins or have aconitase activity, depending upon a [4Fe-4S] cluster located in the protein. High cellular Fe concentrations will cause the [4Fe-4S] cluster to assemble, which will in turn cause the protein to exhibit aconitase activity and bind mRNA with low affinity (Chen et al., 1997). This allows Fe status to determine what properties the protein will exhibit without altering the overall quantity of protein present in the cell.

The addition of a novel 73 amino acid insertion distinguishes IRP2 from IRP1 (Guo et al., 1995a). Iron regulatory protein 2 differs from IRP1 in that it does not exhibit aconitase activity. The abundance of IRP2 is substantially reduced during Fe sufficiency, which is due to increased protein degradation by the proteasome complex (Guo et al., 1995b).

Iron regulatory proteins can be categorized into different pools depending upon their characteristics: 1) a free active pool, 2) an endogenously bound complex which includes an IRP bound to an IRE sequence, or 3) an inactive or low binding affinity protein which includes c-acon and an oxidized form of IRP2 (Barton et al., 1990). For experimental purposes, the total amount of IRP1 can be determined in the presence of high levels of 2-mercaptoethanol (2-ME), which temporarily converts any c-acon into IRP1 (Hentze et al., 1989).

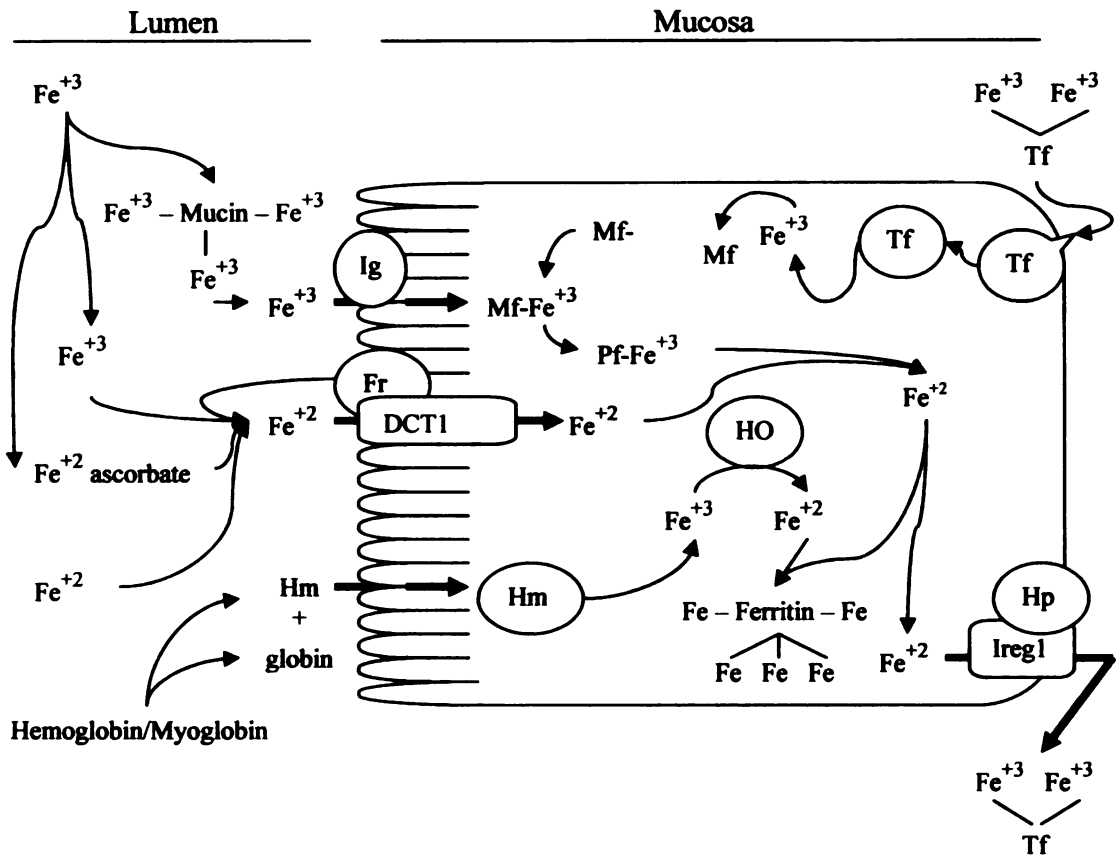
Metabolism

The means by which Fe is taken up by the intestine and transported to tissue is not completely understood. The "mucosal block" theory was proposed by Hahn et al. (1943) and states that an animal absorbs only enough Fe to meet its needs, and the Fe absorbed in excess of the requirement is incorporated into mucosal cell ferritin, where it functions as a "block" against excess assimilation of dietary Fe. Crosby (1963) furthered the hypothesis of intestinal mucosa regulation of Fe homeostasis based on the observation that subsequent to changes in body Fe status, a delay of 2 to 3 d, corresponding to the life span of the enterocyte, was required before a change in Fe absorption occurred. This theory continues to be modified as new insights help elucidate the exact mechanisms of Fe absorption.

Iron homeostasis is largely regulated via absorption because bodily Fe losses are limited. Most of the total Fe present in feces is non-absorbed dietary Fe. An adult male loses approximately 1 mg of Fe daily, mostly in desquamated epithelium and secretions from the gut and skin (Dubach et al., 1955; Weintraub et al., 1965). During a female's reproductive years, daily Fe losses are increased as a result of menstrual bleeding and pregnancy (Cole et al., 1972). The absorption of Fe in animals and humans is affected by: 1) age and Fe status; 2) the amount and chemical form of Fe consumed; 3) the amounts and proportions of various other components of the diet; and 4) the rate of erythrocyte production (Yip and Dallman, 1996). It is generally thought that the two most important factors affecting Fe absorption are Fe stores and erythropoietic demands.

Crichton (2001) divides the process of Fe absorption into three stages: Fe uptake from the intestinal lumen across the brush border; Fe transport across the mucosal cell, associated with storage in the mucosa; and Fe release from the mucosal cell into plasma. The postulated mechanisms of Fe absorption are presented in Figure 4.

Figure 4. Postulated mechanisms of Fe absorption[ab]



[a]Letter abbreviations indicate: Fr (ferrireductase); DCT1 (divalent cation transporter 1); Ig (integrin); Mf (mobilferrin); Pf (paraferritin); Hm (heme); Tf (transferrin); HO (heme oxygenase); Hp (hephaestin); Ireg1 (iron-regulated transporter 1).
[b]Adapted from Conrad et al. (1999) and Crichton (2001).

Although no Fe absorption occurs in the stomach, parietal cells secrete hydrochloric acid, which denatures protein bound Fe and assists in the solubilization and reduction of Fe^{+3} to Fe^{+2} (Beard and Dawson, 1997). Iron is absorbed primarily in the duodenum and upper jejunum (Schumann et al., 1990). The pH level of the duodenum helps maintain Fe in the Fe^{+2} form which is more soluble and readily absorbed than Fe^{+3}. Numerous dietary factors also affect Fe absorption during the intestinal lumen phase. Organic acids, including ascorbic acid (Sayers et al., 1973) and citric acid (Carpenter and Mahoney, 1992), increase nonheme Fe absorption. Amino acids such as histidine, cysteine, and glutathione also increase Fe absorption by providing ligands that prevent polymerization and precipitation of heme and nonheme Fe (Van Campen and Gross, 1969; Layrisse et al., 1984). However, high concentrations of dietary fiber, phytates, tannins, and polyphenols inhibit Fe absorption by forming insoluble chelates (Conrad et al., 1999). Other minerals which have been reported to have an antagonistic effect on Fe absorption when included in excessive concentrations in the diet are Cu (Bradley et al., 1983), Mn (Baker and Halpin, 1991), and Zn (Settlemire and Matrone, 1967).

Following digestion, Fe is present in the intestinal lumen as either heme or nonheme Fe chelates. Within these nonheme Fe chelates, Fe is present in the Fe^{+3} or Fe^{+2} form. Uptake of nonheme Fe chelates by enterocytes is mediated via a series of receptors and binding proteins. It has been proposed that the integrin-mobilferrin system facilitates the absorption of dietary inorganic Fe^{+3} (Conrad et al., 1999). Dietary Fe^{+3} ions first form a chelate with mucin, an intraluminal binding protein that maintains Fe^{+3} in a soluble and available form for absorption. Zinc and Pb have been reported to inhibit chelation of Fe to mucin (Umbreit et al., 1998). The intraluminal chelate then binds with integrin,

located on the apical membrane of the enterocyte, and Fe is subsequently transferred to mobilferrin. It is unclear whether integrin functions to directly transport Fe or serves as an anchor for mobilferrin (Umbreit et al., 1998). Mobilferrin is a cytosolic transport protein that is highly homologous to calreticulin (Conrad et al., 1993). Iron is then delivered by mobilferrin to a protein complex known as paraferritin that contains mobilferrin, integrin, and flavin monoxygenase (Uzel and Conrad, 1998). Paraferritin, via its reductase properties, converts Fe^{+3} to Fe^{+2}, the form required for the synthesis of Fe-containing proteins (Uzel and Conrad, 1998).

Another method of nonheme Fe absorption involves reduction and subsequent transport into the mucosal cell via a transport protein. Nonheme Fe^{+3} is reduced by an apical, membrane bound ferrireductase. McKie et al. (2001) isolated a ferrireductase, duodenal cytochrome *b* (Dcytb), from mouse duodenal mucosa. These authors also reported that expression levels of Dcytb mRNA and protein were regulated by changes in physiological modulators of Fe absorption (McKie et al., 2001). The reduced Fe^{+2} is then transported across the brush border membrane by the divalent metal ion transporter protein, Nramp2, also known as DCT1, which is located primarily in the proximal region of the duodenum (Gruenheid et al., 1995). In addition to Fe^{+2}, Gunshin et al. (1997) noted that Nramp2 exhibits a broad substrate range including Zn^{+2}, Mn^{+2}, Co^{+2}, Cd^{+2}, Cu^{+2}, Ni^{+2}, and Pb^{+2}.

Heme Fe is much more efficiently absorbed than nonheme Fe. Grasbeck et al. (1982) reported that heme receptors were expressed in the duodenal region of the pig intestine. Heme is enzymatically cleaved from Hb in the intestinal lumen and enters the absorptive cell as an intact metalloporphyrin (Callender et al., 1957). After entering the cell, the

enzyme heme oxygenase degrades heme to Fe^{+2}, carbon monoxide, and bilirubin IXa (Raffin et al., 1974). The Fe^{+2} ion subsequently enters the common cytosolic Fe pool and the next steps in the absorption process are similar for heme and nonheme Fe (Beard and Dawson, 1997).

The identification of a duodenal Fe-export protein, Ireg1 (McKie et al., 2001), also referred to as ferroportin (Donovan et al., 2000) and MTP1 (Abboud and Haile, 2000), suggests a method by which Fe is transported across the basolateral membrane of the enterocyte. Working in conjunction with Ireg1 at the basolateral membrane is a Cu-oxidase protein, hephaestin, which promotes release and binding of Fe^{+3} to circulating apotransferrin in plasma (Vulpe et al., 1999).

Researchers have reported that the villi tips of enterocytes in the duodenal region exhibit the greatest uptake of Fe from the lumen in rats (O'Riordan et al., 1997) and adult guinea pigs (Chowrimootoo et al., 1992). The capacity of Fe absorbed from the lumen by mature enterocytes at the villi tips may be a result of the Fe supply to young enterocytes from the blood side (basolateral side) during their proliferation in the crypt region. Uptake of Fe from blood is thought to predominate in the crypt region where immature enterocytes express basolateral TfR (Anderson et al., 1994). These enterocytes then mature as they migrate towards the villi tips. Schumann et al. (1999) reported that ^{59}Fe accumulated in duodenal enterocytes predominantly at the crypt-villus junction 12 h after intravenous injection, while IRP1 activity decreased at this site. After 48 h, the concentration of ^{59}Fe in the upper half of the villi had increased and IRP1 activity had decreased, probably due to migration of ^{59}Fe-containing enterocytes along the crypt-villus axis (Schumann et al., 1999). This suggests that IRPs are involved in the

adaptation of intestinal Fe absorption to changes in Fe stores in the body, possibly by affecting the translation rate of Nramp2 during enterocyte differentiation. In addition, these authors reported that 2 h after enteral Fe administration a decrease in IRP binding and a subsequent decrease in intestinal Fe absorption occurred (Schumann et al., 1999). They suggested that the rapid decrease in IRP binding activity would not provide enough time for sufficient quantities of ferritin to be synthesized to exert a "block" on Fe absorption (Schumann et al., 1999).

Along with the integrin-mobilferrin transport and the heme pathways, the primary method by which nonintestinal cells acquire Fe is via the Tf-TfR pathway. The TfR is composed of two identical subunits each with the capability of binding one Tf molecule (Wada et al., 1979). The number of TfR on the cell surface is dependent upon the intracellular Fe status, cell proliferation state, and metabolic function such as Hb or myoglobin production. Consequently, erythroblasts and reticulocytes exhibit the greatest number of receptors per cell (Iacopetta et al., 1982). The holotransferrin-TfR complex is taken up by TfR mediated endocytosis. Following internalization, Fe is released as a result of the lower pH in the endosome and subsequently reduced to Fe^{+2} via the endosome's reductase properties (Nunez et al., 1990). The Fe^{+2} ion in the endosomal compartment is transported to the cytosol possibly by a H^{+}-ATPase (Li et al., 1994). Depending upon the cellular Fe status and metabolic needs, Fe delivered to the cytoplasm has three possible fates: 1) it can be used for the synthesis of Fe-containing proteins such as Hb; 2) it can be stored as ferritin; or 3) it can regulate the expression of proteins involved in Fe metabolism via IRP binding affinity. The apotransferrin-TfR complex is

packaged along with newly synthesized TfR in the golgi apparatus and then transported to the cell surface where apotransferrin is released (Beard and Dawson, 1997).

The body conserves Fe once it has been absorbed. When erythrocytes are destroyed, the Fe in them is recycled. In men, about 95% of the Fe used in production of new erythrocytes is estimated to come from recycled Fe (Yip et al., 1998). This system of Fe reutilization is an efficient mechanism whereby a constant source of Fe is available daily for synthesis of body Hb.

Deficiency

Iron deficiency is one of the most common nutritional disorders in humans. The sectors which are at the most risk include infants, young children, and women of childbearing age. A National Health and Nutrition Examination survey (2001) reported that the estimated prevalence of Fe deficiency in the United States was greatest among toddlers aged 1 to 2 yr (7%) and adolescent and adult women aged 12 to 49 yr (12%). Iron deficiency is more prevalent in developing countries because of typically lower dietary Fe intakes and a lower Fe bioavailability from the diet. Stoltzfus et al. (2000) reported that tropical regions are especially at risk because Fe deficiency, malaria, and multiple parasite infections coexist. The amount of blood lost varies with the type of parasite and with the number of parasites present (Stoltzfus et al., 2000).

The occurrence of Fe deficiency in ruminants is of little concern due to the high Fe content of pastures and forages and the opportunity for their contamination from Fe in soil. Even so, special attention must be given to prevent Fe deficiency in young preruminant animals that are consuming diets composed primarily of milk or milk products.

Several factors contribute to the potential development of Fe deficiency in young pigs. The hepatic Fe stores of a newborn pig combined with the low Fe concentration in sow's milk are not sufficient to meet the neonate's rapid growth and increase in blood volume. Similar to the suckling phase, the nursery phase is also characterized by rapid growth and increased blood volume. This is especially true in today's swine industry where genetics have been selected for increased growth performance and carcass leanness.

Underwood and Suttle (1999) describe the physiological events induced by Fe deprivation and the resultant subnormal bodily Fe status in four stages. The first stage is depletion, during which liver, kidney, and spleen stores (i.e., ferritin and hemosiderin) are reduced. Blood variables related to Fe status are not altered during the depletion stage. The onset and duration of the depletion period is determined by the abundance of the initial storage pools, primarily in the liver.

The second physiological stage described by Underwood and Suttle (1999) in Fe disorder is deficiency, which is characterized by decreases in plasma Fe (PFe), Hb, hematocrit (Hct), and myoglobin concentrations. A continued decrease in Fe stores is also observed during this stage. These changes are accompanied by an increase in the concentration of Tf in an effort to distribute nonheme Fe to tissues of the body that require Fe.

The third physiological stage described by Underwood and Suttle (1999) is dysfunction. During this stage, functions dependent upon Fe status become rate limiting to particular metabolic pathways. The incorporation of Fe into Hb, myoglobin, and cytochromes are examples of an Fe dependent function.

Disease is the final stage described in Fe deprivation (Underwood and Suttle, 1999). This stage is characterized by clinical symptoms such as a reduction in growth and feed intake, lethargy, and labored breathing or "thumps". These signs are preceded by and largely caused by the development of hypochromic microcytic anemia (Underwood and Suttle, 1999). Amine et al. (1972b) suggested that BW gain is not a sensitive indicator of Fe deficiency because a decrease in growth is only a part of the final stage of Fe disorder. Necropsy of an Fe-deficient animal will reveal clear fluid in body cavities, a pale, soft and dilated heart, an enlarged and fatty liver, and blood with a thin, watery appearance (Zimmerman, 1980).

Toxicity

Iron toxicosis is generally not a concern in animals. Still, caution must be given because of the accidental contributions that can occur during feed manufacturing. Signs of Fe toxicosis include reduced growth and feed intake, diarrhea, hypothermia, and immobility. Most livestock species can tolerate acute exposure to a high concentration of dietary Fe (swine = 3,000; cattle = 1,000; poultry = 1,000; sheep = 500 mg of Fe/kg of diet) and may not exhibit signs of Fe toxicosis (NRC, 1980). However, chronic exposure to slightly lower concentrations may cause liver injury (Underwood and Suttle, 1999). A marginal band of 750 to 1,250 mg of Fe/kg of diet for ruminants and poultry and 2,500 to 3,500 mg of Fe/kg of diet for swine is considered to separate the acceptable from the potentially harmful Fe concentration if the Fe is present in an available form (Underwood and Suttle, 1999).

Campbell (1961) reported that an oral dose of 600 mg of $FeSO_4$/kg of BW to pigs 3- to 10-d of age caused clinical signs of toxicosis within 1 to 3 h of administration. In

growing pigs, excessive dietary Fe concentrations (5,000 mg of Fe/kg of diet or greater) have been reported to cause signs of a P deficiency (Furugouri, 1972). However, O'Donovan et al. (1963) reported that the severity of Fe toxicosis could be reduced by increasing the dietary P concentration.

Hereditary hemochromatosis is one of the most common human genetic disorders in the United States. Approximately one of every 200 to 400 people is affected, while one in 10 is a carrier (CDC, 2001). It is associated with excessive intestinal Fe absorption and the subsequent storage of mass quantities of Fe by various organs including the liver, pancreas, heart, and pituitary gland, ultimately leading to oxidative damage of these organs. Hereditary hemochromatosis is caused by a missense mutation within the Human Factors Engineering (HFE) gene that results in the protein interacting with the TfR (Rolfs et al., 2002). Uptake of Fe by the cell is reduced because of this interaction, which in turn leads to lower but sufficient intracellular Fe concentrations (Riedel et al., 1999). The severity of hereditary hemochromatosis in patients is variable and may be either intensified or minimized by factors such as gender, ethnicity, or dietary nutrients (Niederau et al., 1998).

Assessment of Fe Status

Hemoglobin concentration is a common indicator of Fe status because of the ease of measurement. In swine, a whole blood Hb concentration of 100 g/L is considered adequate, while 80 g/L suggests borderline anemia, and 70 g/L or less indicates anemia (Zimmerman, 1980). In humans, a whole blood Hb concentration less than 120 g/L is used to indicate Fe deficiency (Khusun et al., 1999).

Hematocrit (packed cell volume) is another commonly measured indicator of bodily Fe status and is the proportion, by volume, of the blood that consists of red blood cells. However, Hct is also affected by hydration status of the animal, with dehydration producing a falsely high Hct.

Several factors limit the diagnostic usefulness of PFe measurements. Circulating PFe turns over 10 to 20 times per d, so an Fe atom typically spends no longer than 2 h in plasma (Schreiber, 1989). In addition, PFe exhibits a diurnal variation, with a decrease in concentration in the evening (Schreiber, 1989).

It is generally believed that the most reliable indicator of Fe supply to developing reticulocytes is not the PFe concentration but rather the degree to which circulating Tf is saturated with Fe (Cook et al., 1985). Consequently, PFe concentration should be determined along with measurements of the iron-binding capacity. The result is total iron-binding capacity (TIBC), which represents the sum of endogenous Fe bound to plasma and the additional Fe that can be specifically bound. Many methods measure the unsaturated iron-binding capacity (UIBC) that is the concentration of Fe that can be taken up by the unbound Tf in native plasma (Cook et al., 1985). Unsaturated iron-binding capacity is then added to PFe concentration to determine TIBC.

Serum ferritin determination provides a relatively noninvasive method of assessing Fe status. Several studies have demonstrated that a close correlation exists between serum ferritin concentration and tissue nonheme Fe concentration. A serum ferritin concentration of 12 μg/L or less represents Fe deficiency (Cook et al., 1985).

Requirements

The Fe requirement for various species is presented in Table 1.

Table 1. Iron requirement for various species

Species	Stage of production	Requirement[a]	Reference
Chicken	Broiler	80 mg/kg	NRC (1994)
Beef cattle	All classes	50 mg/kg	NRC (1996)
Dairy cattle	Growing	15 to 43 mg/kg	NRC (2001)
Sheep	All classes	30 to 50 mg/kg	NRC (1985)
Horses	All classes	40 to 50 mg/kg	NRC (1989)
Swine	3 to 10 kg	100 mg/kg	NRC (1998)
Swine	10 to 20 kg	80 mg/kg	NRC (1998)
Swine	20 to 50 kg	60 mg/kg	NRC (1998)
Swine	50 to 80 kg	50 mg/kg	NRC (1998)
Swine	80 to 120 kg	40 mg/kg	NRC (1998)
Swine	Gestation	80 mg/kg	NRC (1998)
Swine	Lactation	80 mg/kg	NRC (1998)
Rats and mice	All classes	35 mg/kg	NRC (1995)
Human	Adults, male	8 to 11 mg/kg	DRI (2001)
Human	Adults, female	8 to 18 mg/kg	DRI (2001)
Human	Adults, pregnant	27 mg/d	DRI (2001)

[a]Expressed as per unit animal feed

Venn et al. (1947) reported that neonatal pigs are born with approximately 50 mg of Fe, primarily as Hb. Researchers were not able to increase placental transfer of Fe to fetuses by feeding a high dietary Fe concentration (Brady et al., 1978) or parenteral administration of Fe dextran (Ducsay et al., 1984) to sows during gestation. Braude et al. (1962) determined that approximately 90% of the Fe requirement during the first few wk of life is utilized for Hb synthesis. Consequently, they estimated that a pig must retain 21 mg of Fe/kg BW gain in order to maintain a satisfactory Hb and storage Fe concentration during the suckling phase (Braude et al., 1962). Other researchers have estimated that a suckling pig must retain 7 to 16 mg of Fe daily to meet its requirement (Venn et al., 1947). Unfortunately, sow's milk, which is the primary source of nutrients for suckling pigs, contains an average of only 1 mg of Fe/L (Brady et al., 1978). Although these authors were able to elevate the Fe concentration in sow's milk by supplementing 3,000 mg of Fe-proteinate/kg of diet, the increase was not sufficient to maintain adequate Hb production (700 g/L or less) in suckling pigs not receiving an exogenous Fe source (Brady et al., 1978). Talbot and Swenson (1970) reported that adequate Fe (150 mg of Fe) is necessary or erythrocyte volume decreases by two wk of age to almost one-half of the erythrocyte volume at birth, and in this study was maintained at this low value (approximately 15 ml/kg of BW) until the conclusion of a six wk study. Thus, the use of an exogenous source of Fe to prevent Fe deficiency in neonatal pigs has been well documented (Maner et al., 1959; Ullrey et al., 1959; Pollmann et al., 1983). The most common and effective method of providing supplemental Fe in the swine industry is by an i.m injection of 100 to 200 mg of Fe dextran. Access to the sow's feed and feces will

also provide Fe to the young pig; however, this should not serve as the primary source of exogenous Fe.

The NRC (1998) postweaning dietary Fe requirement, 80 mg of Fe/kg of diet, is based on early experiments conducted by Pickett et al. (1960) who reported reduced growth in pigs weaned at 10- to 14-d of age and fed diets containing 60 mg of added Fe/kg of diet. These pigs also had decreased Hb and Hct when fed diets containing 80 mg of added Fe/kg of diet (Pickett et al., 1960). Most of the postweaning Fe requirement is thought to be met by the Fe provided by common feed ingredients. However, the bioavailability of Fe from different sources varies greatly (Kornegay, 1972; Deming and Czarnecki-Maulden, 1989b) and is influenced by such factors as Fe status of the animal, dietary Fe concentration, and various nutritional and nonnutritional elements in the diet. Therefore, a commercial base mineral premix containing 15,000 to 20,000 mg of Fe/kg of premix is typically supplemented to the diet.

The Fe requirement, as a percentage of the diet, decreases as BW increases throughout the stages of swine production. The Fe requirement decreases because blood volume relative to BW and weight gain relative to feed consumption decrease with growth. As a result, the occurrence of Fe deficiency in later stages of swine production is rare.

In conclusions, there is a need to reevaluate the dietary Fe requirement using genetics, diets, and management practices representative of today's swine industry. As well, by utilizing laboratory techniques that are more-sensitive to Fe state; we can gain a better understanding of a young pig's Fe status during this rapid growth phase.

CHAPTER TWO

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CHAPTER TWO

EFFECTS OF DIETARY IRON SUPPLEMENTATION ON GROWTH PERFORMANCE, HEMATOLOGICAL STATUS, AND WHOLE-BODY MINERAL CONCENTRATIONS OF NURSERY PIGS

Abstract: An experiment was conducted to evaluate the effects of supplementing increasing concentrations of Fe to the diet of nursery pigs on growth performance and indices of hematological and mineral status. Pigs (n = 225; 6.5 kg; 19 ± 3 d) were randomly allotted by BW, litter, and gender to one of five dietary treatments (5 pigs per pen; 9 pens per treatment). Basal diets for each phase (Phase 1: d 0 to 7; Phase 2: d 7 to 21; Phase 3: d 21 to 35) were formulated to contain minimal Fe concentration and then supplemented with 0, 25, 50, 100, and 150 mg Fe/kg of diet (as-fed basis) from ferrous sulfate. Three pigs per pen (n = 135) were chosen and bled throughout (d 0, 7, 21, and 35) to determine hemoglobin (Hb), hematocrit (Hct), transferrin (Tf), and plasma Fe (PFe). Also, pigs (n = 5; 5.9 kg; 19 ± 3 d) from the contemporary group were killed at d 0 to establish baseline (BL) and 30 pigs (6 pigs/treatment) were killed at d 35 to determine whole-body and liver mineral concentrations. The improvements in growth performance during Phase 2 (ADG, linear, $P = 0.036$; ADFI, linear, $P = 0.096$; G:F, quadratic, $P = 0.075$) were of sufficient magnitude that dietary treatments tended to increase ADG (linear, $P = 0.084$), ADFI (quadratic, $P = 0.095$), and G:F (quadratic, $P = 0.107$) for the 35-d experiment. Hematological variables were not affected until d 21, at which time dietary Fe supplementation resulted in a linear increase ($P < 0.050$) in Hb, Hct, and PFe. This linear increase ($P = 0.001$) was maintained until d 35 of the experiment; however, dietary treatments resulted in a linear decrease ($P = 0.004$) in Tf on

d 35. Whole-body Fe concentration increased (linear, $P = 0.003$) in pigs due to increasing dietary Fe concentrations. Moreover, pigs fed for 35 d had greater ($P < 0.010$) whole-body Fe, Zn, Mg, Mn, Ca, and P concentrations and lower ($P = 0.001$) whole-body Cu concentration than BL. Hepatic Fe concentration increased (linear, $P = 0.001$) in pigs due to dietary treatments; however, the hepatic Fe concentration of all pigs killed on d 35 was lower ($P = 0.001$) than the BL pigs. Results suggest that Fe contributed by feed ingredients was not sufficient to maintain indices of Fe status. The decrease in Fe stores of the pigs was not severe enough to reduce growth performance. Even so, the lessening of a pig's Fe stores during this rapid growth period may result in the occurrence of anemia during the subsequent grower and finisher periods.

Introduction

The most common potential mineral deficiency in swine is Fe deficiency. Several factors contribute to this deficiency in nursing pigs. The hepatic Fe stores of a newborn pig combined with the low Fe concentration in sow's milk are not sufficient to meet the pig's rapid growth and increase in blood volume. Consequently, the use of an exogenous source of Fe to prevent Fe deficiency in neonatal pigs has been well documented (Ullrey et al., 1959; Kernkamp et al., 1962). Similar to the suckling phase, the nursery phase is also characterized by increased blood volume and rapid growth. This is especially true in the swine industry today with genetics that have been selected for increased growth performance and carcass leanness. The NRC (1998) postweaning dietary Fe requirement, 80 mg/kg, is based on early experiments conducted by Pickett et al. (1960) who reported decreased growth in pigs weaned at 10 to 14 d of age and fed diets containing 60 mg added Fe/kg diet and decreased hemoglobin (Hb) and hematocrit (Hct) in pigs fed diets containing 80 mg added Fe/kg diet. The Fe provided by common feed ingredients may meet most of the postweaning Fe requirement. However, the bioavailability of Fe from different sources varies greatly (Kornegay, 1972; Deming and Czarnecki-Maulden, 1989a) and is influenced by such factors as Fe status of the animal, dietary Fe concentration, and various nutritional and nonnutritional elements within the diet.

The experimental objective was to evaluate the effects of increasing concentrations of supplemental ferrous sulfate in diets using ingredients commonly included in commercial diets, but still providing minimal Fe to the basal diet on nursery pig growth performance, hematological status, and whole-body mineral concentrations and chemical composition.

Materials and Methods

Animal Use and Care

This experiment was conducted at the Michigan State University Swine Teaching and Research Facility. Use of animals in this experiment was approved by the All-University Committee on Animal Use and Care at Michigan State University (Animal Use Form No. 12/02-164-00).

Animals, Diets, and Housing

Two hundred twenty-five pigs (Duroc x Landrace – Yorkshire, Landrace x Yorkshire, and Yorkshire) were weaned at 19 ± 3 d and used during a 35-d feeding experiment. Before initiating the experiment, pigs were managed according to facility standard operating procedures and received 200 mg of Fe via i.m. injection of Fe dextran (Phoenix Pharmaceutical, Inc., Saint Joseph, MO) at 1 to 2 d of age. At the start of the experiment, pigs were blocked on the basis of initial BW (mean = 6.5 kg), while equalizing ancestry and gender across treatments, to five dietary treatments in a randomized complete block design. There were nine replicate pens per treatment with five pigs per pen.

Basal diets (Table 1) for each dietary phase (Phase 1: d 0 to 7; Phase 2: d 7 to 21; Phase 3: d 21 to 35) were formulated based on previous analysis of similar dietary ingredients and, when necessary, on values published in the NRC (1998) feed composition tables. Feed ingredients commonly included in commercial nursery diets yet containing a low concentration of Fe were used. To minimize the Fe contribution from dietary Ca and P sources, experimental diets were formulated using calcium sulfate and sodium phosphate, respectively, because they are known to contain minimal Fe (NRC, 1998). To limit the Fe concentration in the basal diets, the base mineral mix used in this experiment contained minimal Fe (729 mg/kg, as-fed basis) in comparison with typical

commercial base mineral mixes which contain 15,000 to 20,000 mg Fe/kg. Dietary treatments were obtained by supplementing the basal diets with 0, 25, 50, 100, and 150 mg Fe per kg of diet (as-fed basis) from ferrous sulfate monohydrate ($FeSO_4 \cdot H_2O$), a highly available Fe source (Harmon et al., 1967; Miller, 1978). Complexity of the diet changed with phases to meet or exceed NRC (1998) nutrient recommendations, excluding Fe, and to satisfy changes in digestive capabilities of the weanling pig. Phase 1 and Phase 2 diets were fed in pelleted form and consisted of highly digestible protein and carbohydrate sources, whereas Phase 3 diets were typical corn-soybean meal-based that were fed in meal form.

Table 1. Composition of basal diets (as-fed basis)

	Diets		
Ingredient, %	Phase 1 (d 0 to 7)	Phase 2 (d 7 to 21)	Phase 3 (d 21 to 35)
Corn[a]	28.23	45.19	59.26
Soybean meal (48% CP)	10.21	20.29	30.70
Whey, spray dried	20.00	10.00	-
Lactose	10.00	5.00	-
Protein plasma	4.00	2.00	-
Egg, spray dried	8.00	4.00	-
Skim milk powder	6.00	2.00	-
Potato protein	5.00	2.00	-
L-Lysine HCl	-	0.15	0.15
Sodium phosphate, monobasic	0.94	1.26	1.49
Calcium sulfate, dihydrate	2.23	2.72	3.08
Sodium chloride	-	-	0.20
Zinc oxide	0.28	0.28	0.01
Copper sulfate	0.01	0.01	0.01
Mineral premix[b]	0.50	0.50	0.50
Vitamin premix[c]	0.60	0.60	0.60
Soybean oil	4.00	4.00	4.00
Analyzed composition			
CP, %	25.98	24.92	26.30
Lysine, %	1.64	1.35	1.32
Ca, %	1.14	1.28	1.08
P, %	0.73	0.75	0.71
Cu, mg/kg	42.90	51.90	14.10
Fe, mg/kg	189.00	223.80	97.80
Mg, mg/kg	2,292.20	2,909.40	1,717.60
Mn, mg/kg	41.54	58.25	27.92
Zn, mg/kg	1,755.30	1,946.50	107.50

[a]Corn was replaced by ferrous sulfate (wt/wt) to provide supplemental dietary Fe concentrations of 0, 25, 50, 100, and 150 mg/kg.

[b]Provided the following per kilogram of diet: 0.14 mg of I as ethylenediamine dihydroiodide; 3.60 mg of Fe as $FeSO_4$; 2.00 mg of Mn as $MnSO_4$; and 0.30 mg of Se as Na_2SeO_3.

[c]Provided the following per kilogram of diet: 5,511 IU of vitamin A; 551 IU of vitamin D_3; 66 IU of vitamin E; 13.2 mg of vitamin K activity; 4.40 mg of menadione; 36 μg of vitamin B_{12}; 4.40 mg of riboflavin; 17.6 mg of d-panthothenic acid; 26.5 mg of niacin; 1.10 mg of thiamine; and 0.99 mg of pyridoxine.

Pigs were housed in a temperature-controlled nursery room (initial room temperature was maintained at 29°C and decreased by 1°C weekly until the room temperature reached 24°C) and grouped in pens with solid hardened steel rod flooring (DPC Agri Systems, Delphi, IN). Each pen provided 2.23 m^2 of space and contained a three-hole, stainless steel feeder and one nipple waterer that allowed for ad libitum access to feed and water throughout the experiment. The mean Fe concentration in drinking water was 0.12 mg/L and exogenous Fe contamination from environmental surroundings was avoided insofar as possible.

Performance, Blood, Tissue, and Whole-body Collection

Pig weights and pen feed disappearance were determined for each dietary phase and utilized in the determination of ADG, ADFI, and G:F.

Initially (d 0), three pigs were randomly chosen from each pen (n = 135) and blood samples were collected. The same 135 pigs were bled again on d 7, 21, and 35. Blood samples were drawn by jugular venipuncture into 10-mL heparinized (143 USP units of sodium heparin per tube) Vacutainer (Becton Dickinson, Franklin Lakes, NJ) tubes with 21 gauge, 3.81-cm needles. An aliquot of whole blood was transferred into a polypropylene tube and stored on ice until Hb and Hct analysis could be performed later that day. The remaining blood was centrifuged at 2,000 x g, 4°C, for 10 min (Beckman GS-6KR, Palo Alto, CA). Plasma was collected into polypropylene tubes and stored at -80°C until mineral and transferrin (Tf) analysis could be performed.

At the conclusion of the experiment, one pig per pen was randomly chosen from 30 pens (6 pigs per treatment; mean BW = 18.4 kg; 54 ± 3 d) and killed via cardiac injection of sodium pentobarbital (87 mg/kg of BW). Liver samples were excised and stored in

Whirl-Pak bags (Nasco, Fort Atkinson, WI) at -80°C until mineral analysis could be performed. The remaining whole-body of each pig was frozen and ground (Autio 801GH, Autio Company Inc., Astoria, OR) twice through a 3.2-mm aperture plate, mixed, and subsampled. Subsamples were freeze-dried (Tri-Philizer MP, FTS Systems Inc., Stone Ridge, NY) and further processed to reduce particle size by submersion in liquid nitrogen and blending in a 1.5 L stainless steel blender (Waring Products Co., New Hartford, CT). Subsamples were then stored in Whirl-Pak bags until analysis for minerals and chemical composition. Additionally, five pigs from the original contemporary group (mean BW = 5.9 kg; 19 ± 3 d) were killed at the initiation of the study. Liver and whole-body samples were collected in a similar manner as that previously described to establish baseline (BL) response criteria for mineral concentrations and chemical composition.

Laboratory Analysis

Mineral Analysis. Feed, liver, and whole-body samples were prepared for mineral analysis via microwave digestion (model MARS-5, CEM, Matthews, NC) as described by Shaw et al. (2002). Calcium, Cu, Fe, Mn, Mg, and Zn analyses were conducted by flame atomic absorption spectrophotometry (Unicam 989, Thermo Elemental Corp., Franklin, MA), and P concentration was determined (Gomori, 1942) using a DU 7400 spectrophotometer (Beckman, Palo Alto, CA). All analyses were performed in duplicate and feed, liver, and whole-body mineral concentrations were reported on an as-fed, fresh, and DM basis, respectively.

Plasma samples were deproteinized by the addition of trichloroacetic acid for Cu (PCu), Zn (PZn), and Fe (PFe) analysis. For PCu and PZn analysis, plasma was diluted

1:4 with 12.5% trichloroacetic acid, mixed, incubated at room temperature for 10 min, and centrifuged (2,000 x g, 4°C, and 15 min). Plasma Fe was diluted 1:3 with 20.0% trichloroacetic acid, mixed, incubated at 90°C for 15 min, and centrifuged at 2,000 x g, 4°C, for 10 min (Olson and Hamlin, 1969). The supernatant fraction was transferred to a clean polypropylene tube and subsequently evaluated using a flame atomic absorption spectrophotometer.

Instrument accuracy for all mineral analyses was established using bovine liver standard (1577b; NIST, Gaithersburg, MD). All glassware used in the mineral analyses was soaked in 30% nitric acid for at least 12 h and rinsed five times with double-deionized water.

Hemoglobin and Hematocrit Analysis. Hemoglobin and its derivatives in whole blood were converted to cyanmethemoglobin and read at 540 nm using a spectrophotometer. Hematocrit was determined by the microhematocrit method (INACG, 1985).

Transferrin Analysis. Porcine plasma Tf concentration was determined by an ELISA at room temperature. Each well of a 96-well Nunc Maxisorp "C"-bottomed microtiter plate (Nalge Nunc International, Rochester, NY) was coated with 0.1 mL of a 1:100 dilution of Affinity Purified Pig Tf Antibody (Bethyl Laboratories Inc., Montgomery, TX) and allowed to incubate for 60 min at room temperature. Wells were then rinsed three times with wash solution (50 mM Tris buffered saline, 0.05% Tween 20, pH 8.0, Sigma-Aldrich Co., St. Louis, MO) and blotted dry. Next, 0.2 mL of postcoat solution (50 mM Tris buffered saline, 1% BSA, pH 8.0, Sigma-Aldrich Co.) was applied to each well and incubated for 30 min. Wells were again washed three times with wash solution

and blotted dry. Pig Reference Plasma (Tf = 50 g/L, Bethyl Laboratories Inc.) was diluted to make the Tf standard increments (19.5 to 1,250.0 ng/mL) and plasma samples, standards, and a control plasma sample were applied in duplicate using 0.1 mL per well. Microtiter plates were then incubated for 60 min, rinsed five times with wash solution, and blotted dry. Then 0.1 mL of Horse Radish Peroxidase Conjugated Pig Tf Antibody (Bethyl Laboratories Inc.) was added to all wells and incubated for 60 min, rinsed five times with wash solution, and blotted dry. Next, 0.1 mL of enzyme substrate (tetramethylbenzidine peroxidase substrate and peroxidase solution B, vol/vol; Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added to all wells and incubated until the color developed sufficiently for the 1,250 ng/mL standard to have an optical density reading greater than 1.6 (approximately 20 min). Finally, 0.1 mL of 2 M sulfuric acid was added to each well to stop the reaction. Results were read at 450 nm using a SpectraMax 340 microtiter reading spectrophotometer (Molecular Devices Corp., Sunnyvale, CA). A four-parameter curve was used for the fit of the slope of the standards. Plasma unknowns were plotted against the standard curve and the concentration (ng/mL) was reported.

Whole-body Chemical Composition. Whole-body samples were analyzed for protein, lipid, and ash composition by standard AOAC (1998) methods utilizing a combustion instrument (FP-2000, LECO Corp., St. Joseph, MI), soxhlet extraction, and a muffle furnace (Type 30400, Thermolyne Subsidiary of Sybron, Rochester, NY), respectively.

Statistical Analysis

Performance (ADG, ADFI, G:F) data were analyzed as a randomized complete block design using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). The model

included the effects of block (replication), treatment, and block x treatment (error) with block considered a random effect. The effects of increasing dietary concentrations of supplemental Fe were partitioned into linear and curvilinear components using orthogonal polynomial contrasts. Due to unequally spaced dietary concentrations of supplemental Fe, coefficients were derived using the integrative matrix language (PROC IML) procedures of SAS. Pen was the experimental unit for analysis of performance data. For tissue and whole-body data, analyses were performed using the MIXED procedures of SAS with individual pig as the experimental unit. Orthogonal polynomial contrasts were used to test for linear and quadratic effects of dietary Fe supplementation. One preplanned orthogonal comparison was made: BL pigs vs. all Fe treatments. In addition, data for blood variables were analyzed using the MIXED model methodology of SAS for analysis of repeated measure data. The subject for the repeated measures on d 7, 21, and 35 was individual pig within pen x treatment and d-0 Hb, Hct, Tf, and PFe values were used as covariates for their respective individual analyses. Differences were considered significant at the level of $P < 0.05$ and highly significant at the level of $P < 0.01$.

Results

The basal diets contained 189.0, 223.8, and 97.8 mg of Fe/kg (as-fed basis) for Phase 1, 2, and 3, respectively. The analyzed concentrations of supplemental Fe for the five dietary treatments in each phase were within 15% of their targeted values (data not shown).

The effects of increasing concentrations of supplemental Fe on pig performance are shown in Table 2. Dietary treatments did not affect ($P > 0.100$) growth performance

during Phase 1 and 3. However, increasing dietary Fe concentrations resulted in a linear increase ($P = 0.036$) in ADG during Phase 2. Also during Phase 2, dietary Fe supplementation tended to increase ADFI (linear, $P = 0.096$) and improve G:F (quadratic, $P = 0.075$). Overall, increasing concentrations of supplemental Fe tended to increase ADG (linear, $P = 0.084$), ADFI (quadratic, $P = 0.095$), and improve G:F (quadratic, $P = 0.107$).

Table 2. Effects of dietary Fe supplementation on nursery pig growth performance[a]

Item[c]	Supplemental Fe, mg/kg of diet					SEM	*P*-value[b]	
	0	25	50	100	150		Linear	Quad
Phase 1, d 0 to 7								
ADG, g	115	121	115	136	111	7	0.995	0.250
ADFI, g	158	165	154	176	151	7	0.869	0.221
G:F, g/kg	730	729	719	775	715	24	0.931	0.651
Phase 2, d 7 to 21								
ADG, g	290	291	310	311	323	7	0.036	0.821
ADFI, g	393	406	417	442	423	9	0.096	0.218
G:F, g/kg	736	717	739	706	764	8	0.277	0.075
Phase 3, d 21 to 35								
ADG, g	433	452	472	452	471	9	0.156	0.627
ADFI, g	702	740	794	750	776	15	0.192	0.291
G:F, g/kg	620	601	611	605	633	8	0.371	0.182
Overall, d 0 to 35								
ADG, g	312	321	335	332	338	13	0.084	0.435
ADFI, g	470	496	507	512	498	22	0.223	0.095
G:F, g/kg	667	657	662	652	674	11	0.556	0.107

[a]Data are least squares means (n = 9 per treatment).

[b]Linear and quadratic effects of increasing Fe concentration.

[c]ADFI reported on an as-fed basis.

There were no differences ($P > 0.100$) in blood response criteria at the start of the experiment (d 0). In general, blood response criteria (Table 3) were not affected by dietary addition of Fe until d 21 of the 35-d feeding experiment. Following completion of Phase 2 (d 21), pigs fed diets containing increasing concentrations of supplemental Fe had a linear increase ($P = 0.001$) in Hb concentration. This linear increase ($P = 0.001$) in mean Hb concentration in response to dietary treatment was maintained until the end of Phase 3 (d 35). Similar to Hb, pigs fed diets supplemented with increasing concentrations of Fe had a linear increase ($P = 0.004$) in Hct on d 21. This linear increase ($P = 0.001$) in Hct was again noted on d 35. Plasma Fe exhibited a linear increase in pigs due to dietary treatments on d 21 ($P = 0.033$) and 35 ($P = 0.001$). On d 35, plasma Tf concentration of pigs fed increasing supplemental dietary Fe concentrations decreased in a linear manner ($P = 0.004$).

No differences in PCu and PZn concentration due to increasing concentrations of supplemental Fe were observed during the 35-d feeding experiment (data not shown).

Table 3. Effects of dietary Fe supplementation on nursery pig hematological status[a]

	Supplemental Fe, mg/kg of diet						*P*-value[b]	
Item	0	25	50	100	150	SEM	Linear	Quad
Hemoglobin, g/L								
d 7	108.49	109.12	109.03	110.31	109.34	2.32	0.710	0.740
d 21	95.55	97.17	101.34	103.18	106.61	2.32	0.001	0.633
d 35	107.01	107.12	117.13	117.79	118.48	2.32	0.001	0.070
Hematocrit, %								
d 7	42.95	42.95	42.73	43.59	43.10	0.36	0.689	0.867
d 21	39.63	39.50	40.44	41.78	42.05	0.34	0.004	0.783
d 35	42.74	43.00	45.93	46.49	46.20	0.38	0.001	0.057
Plasma Fe, mg/L								
d 7	1.10	1.16	0.98	1.23	1.04	0.07	0.913	0.670
d 21	1.17	1.14	1.29	1.17	1.52	0.08	0.033	0.302
d 35	1.27	1.49	1.73	1.74	1.79	0.08	0.001	0.068
Plasma Transferrin, g/L								
d 35	44.66	47.13	39.38	39.76	39.08	0.42	0.004	0.292

[a]Data are least squares means (n = 27 per treatment). Blood levels on d 0 were: hemoglobin = 110 g/L (SEM = 1.10); hematocrit = 42.94% (SEM = 0.43); plasma Fe = 0.45 mg/L (SEM = 0.01); plasma transferrin = 52.20 g/L (SEM = 1.00).

[b]Linear and quadratic effects of increasing Fe concentration.

The effects of dietary Fe supplementation on liver mineral concentrations of nursery pigs are presented in Table 4. Mean baseline liver Fe concentration on d 0 was greater (P = 0.001) than pigs fed any of the dietary treatments for 35 d. A dietary effect was also observed as pigs fed diets containing increasing concentrations of supplemental Fe had a linear increase (P = 0.001) in liver Fe concentration. Furthermore, pigs fed for 35 d had lower hepatic Cu, Zn, and Mg (P = 0.001) concentrations in contrast to BL. Finally, no differences were observed in hepatic Mn, Ca, and P concentrations.

Table 4. Effects of dietary Fe supplementation on nursery pig liver mineral concentrations (wet basis)[a]

			Supplemental Fe, mg/kg of diet						*P*-value[b]		
Mineral[c]	BL	SE[c]	0	25	50	100	150	SE[c]	BL vs. Fe	Linear	Quad
DM, %	25.5		27.8	26.9	27.2	27.6	27.4				
Fe, mg/kg	247	12	35	38	86	123	113	8	0.001	0.001	0.003
Cu, mg/kg	82.5	1.9	12.0	12.6	11.9	11.5	12.0	1.5	0.001	0.811	0.875
Zn, mg/kg	87.8	6.5	42.8	42.9	45.2	47.6	56.1	5.3	0.001	0.090	0.581
Mg, mg/kg	210	8	184	186	187	195	186	3	0.001	0.333	0.123
Mn, mg/kg	2.44	0.36	2.88	3.28	2.96	2.77	2.97	0.33	0.178	0.722	0.936
Ca, mg/kg	55.8	2.6	51.3	54.0	56.6	53.4	52.3	2.4	0.421	0.916	0.180
P, g/kg	3.44	0.07	3.43	3.8	3.39	3.31	3.54	0.06	0.678	0.317	0.038

[a]Data are least squares means: BL = Baseline (n = 5; 5.9 kg; 19 ± 3 d). For other data, n = 6 per treatment (18.4 kg; 54 ± 3 d).

[b]Preplanned orthogonal comparison between BL pigs vs. all Fe treatments (0 to 150 mg of added Fe/kg of diet). Linear and quadratic effects of increasing Fe concentration.

[c]Standard errors are presented separately for BL or each dietary treatment because of different numbers of observations.

Whole-body mineral concentrations of nursery pigs are shown in Table 5. An increase in supplemental dietary Fe resulted in a linear increase ($P = 0.003$) in whole-body Fe concentration. Also, pigs fed diets for 35 d had greater ($P = 0.016$) whole-body Fe concentration than the BL pigs. No differences ($P > 0.100$) were observed in whole-body Cu, Zn, Mg, Mn, Ca, and P concentrations due to increasing concentrations of supplemental Fe; however, pigs fed for 35 d had lower Cu and greater Zn, Mg, Mn, Ca, and P whole-body concentrations when compared with BL ($P = 0.001$).

Whole-body chemical composition of nursery pigs is also presented in Table 5. Baseline pigs had a greater percentage of lipid and a smaller percentage of water and ash ($P = 0.001$) than did pigs fed for 35 d. No differences ($P > 0.100$) were observed in whole-body chemical composition due to dietary Fe supplementation.

Table 5. Effects of dietary Fe supplementation on nursery pig whole-body mineral concentrations (DM basis) and chemical composition[a]

			Supplemental Fe, mg/kg of diet						*P*-value[b]		
Item	BL	SE[c]	0	25	50	100	150	SE[c]	BL vs. Fe	Linear	Quad
Mineral[c]											
Fe, mg/kg	139	7	142	152	162	165	171	7	0.016	0.003	0.327
Cu, mg/kg	9.5	0.5	6.2	6.1	6.0	5.4	5.8	0.4	0.001	0.270	0.526
Zn, mg/kg	59.7	2.9	74.4	71.5	71.5	70.5	73.5	2.7	0.001	0.872	0.258
Mg, mg/kg	730	27	982	968	955	999	974	25	0.001	0.777	0.950
Mn, mg/kg	0.14	0.26	3.38	3.72	3.32	3.53	3.44	0.24	0.001	0.917	0.846
Ca, g/kg	16.7	1.1	26.4	23.7	24.9	25.5	25.1	1.0	0.001	0.965	0.484
P, g/kg	11.5	0.5	15.5	15.0	14.7	15.3	15.0	0.5	0.001	0.719	0.533
Chemical composition											
Water, %	67.3	0.5	71.6	71.6	70.7	71.1	71.0	0.4	0.001	0.283	0.470
Protein, %	15.8	0.2	16.0	15.7	16.1	16.0	15.9	0.2	0.399	0.923	0.940
Lipid, %	15.1	0.5	9.7	10.0	10.4	10.1	10.5	0.5	0.001	0.271	0.798
Ash, %	2.3	0.1	2.8	2.8	2.7	2.8	2.7	0.1	0.001	0.293	0.219

[a]Data are least squares means: BL = Baseline (n = 5; 5.9 kg; 19 ± 3 d). For other data, n = 6 per treatment (18.4 kg; 54 ± 3 d).
[b]Preplanned orthogonal comparison between BL pigs vs. all Fe treatments (0 to 150 mg of added Fe/kg of diet). Linear and quadratic effects of increasing Fe concentration.
[c]Standard errors are presented separately for BL or each dietary treatment because of different numbers of observations.

Discussion

Underwood and Suttle (1999) describe the physiological events induced by Fe deprivation and resultant subnormal bodily Fe status in four stages. The first stage is depletion, during which liver, kidney, and spleen stores (i.e., ferritin and hemosiderin) are decreased. Blood variables related to Fe status are not altered during depletion. The onset and duration of the depletion period is determined by the abundance of the initial storage pools, primarily in the liver. For the present experiment, mean BL liver Fe concentration (232 mg/kg) was within the normal range for the nursery pig reported by Underwood and Suttle (1999). Following completion of the 35-d feeding experiment, liver Fe stores were decreased in pigs fed all dietary treatments. The hepatic Fe concentration of pigs fed diets with 0 or 25 mg added Fe/kg decreased to within the marginal range separating deficient from adequate hepatic Fe concentrations reported by Underwood and Suttle (1999). Recent work by Yu et al. (2000) noted a linear decrease in liver ferritin, nonheme Fe, and total Fe as dietary Fe supplementation decreased from 120 to 0 mg Fe/kg. A linear decrease in liver ferritin, nonheme, hemosiderin, and total Fe was also reported by Furugouri (1972) when dietary Fe decreased.

The second physiological stage described by Underwood and Suttle (1999) in Fe disorder is deficiency that is characterized by decreases in PFe, Hb, Hct, and myoglobin concentrations. A continued decrease in Fe stores is also observed during this stage. These changes are accompanied by increases in the concentration of the principal Fe transport protein, Tf, in an effort by the body to distribute nonheme Fe to the Fe-requiring tissues.

A whole blood Hb concentration of 100 g/L is considered adequate, while 80 g/L suggests borderline anemia, and 70 g/L or less indicates anemia (Zimmerman, 1980). At the start of this study, pigs in all dietary treatments had a mean Hb concentration of 110 g/L or greater. Mean Hb concentrations did not decrease until d 21; however, Hb concentration for all dietary treatments remained above the borderline anemia concentration (80 g/L). The linear decrease in Hb concentration noted in this experiment is in agreement with the results of Furugouri (1972) and Hedges and Kornegay (1973) who reported linear decreases in Hb concentration with decreasing dietary Fe concentrations. An increase in mean Hb concentration was observed in all treatments during Phase 3 of the current study even though the basal diet (corn-soybean meal-based) fed during Phase 3 had a lower Fe concentration by analysis (98 mg/kg) than the Phase 1 (189 mg/kg) or Phase 2 (224 mg/kg) diets. Harmon et al. (1968) reported that the percentage of dietary Fe retained by the body increases as dietary Fe concentration decreases.

Hematocrit is the proportion, by volume, of the blood that consists of red blood cells and is expressed as a percentage. Initial mean Hct for pigs in this study were comparable to values reported by Talbot and Swenson (1970), but greater than those reported by Miller et al. (1961) for similar age pigs. Hematocrit is affected by the hydration status of the animal, with dehydration producing a falsely high Hct. Stress at weaning can influence the hydration state of an animal. Hematocrit in pigs, similar to Hb, responded to dietary treatments with a linear increase on d 21. These results agree with those of Hedges and Kornegay (1973) and Dove and Haydon (1991) who reported increased percentages of Hct after 28 d as dietary Fe concentration increased. Yu et al. (2000)

reported a linear increase in Hct as dietary Fe supplementation increased up to 120 mg Fe/kg.

Several factors limit the diagnostic usefulness of PFe measurements. Circulating PFe turns over 10 to 20 times daily, so an Fe atom typically spends no longer than 2 h in plasma (Schreiber, 1989). In addition, PFe exhibits a diurnal variation, with a decrease in concentration in the evening (Schreiber, 1989). Initial (d 0) PFe values were lower than values reported by Smith (1989); however, by d 7 they had increased to within the range (0.60 to 1.49 mg/L) reported by Underwood and Suttle (1999) for the nursery pig. The observed linear increase at d 35 in PFe due to increasing concentrations of dietary Fe in this experiment are similar to results reported by Yu et al. (2000).

A key facilitator in the maintenance of Fe homeostasis is the plasma glycoprotein, Tf, which is the primary means of interorgan transport of nonheme Fe. Elevated Tf concentration is associated with an increase in Fe absorption from the gut or mobilization of Fe from tissues stores. The abundance of plasma Tf is inversely related to Fe status in rats (Morton and Tavill, 1977; Zakin, 1992) and chicks (McKnight et al., 1980). To our knowledge there are no published values for plasma Tf concentration in swine. The high mean plasma Tf concentration on d 0 may be indicative of the erythropoietic demand by the pig at this stage of development. Talbot and Swenson (1970) reported that during the first 6 wk of life the erythrocyte volume per kg of BW was greatest in pigs at 3 wk of age given an Fe supplement. On d 35, the observed linear decrease in plasma Tf concentration suggests a greater need to transport nonheme Fe due to demand by Fe-dependent tissue in pigs fed lower dietary Fe (0 and 25 mg of added Fe/kg). Other researchers have shown that an increase in plasma Tf concentration in chicks (McKnight

et al., 1980) and rats (Idzerda et al., 1986) is associated with a simultaneous increase in Tf gene transcription in the liver, the primary site of Tf synthesis.

The third physiological stage described by Underwood and Suttle (1999) is dysfunction. During this stage, Fe-dependent functions, including the incorporation of Fe into the heme proteins Hb, myoglobin, and cytochrome c oxidase, become rate limiting to particular metabolic pathways. The principal use of Fe in the body is incorporation into Hb, accounting for 60 to 80% of body Fe (Miller et al., 1981). Because Hb functions to carry oxygen from lungs to other tissues, it is commonly used as an indicator of the pig's Fe status. Myoglobin accounts for 10% of body Fe and is predominantly located in muscle cells where it functions to bind oxygen (Clydesdale and Francis, 1971). Although myoglobin was not determined in this study, Hagler et al. (1981) reported that rats fed an Fe-deficient diet had decreased muscle myoglobin concentration.

Disease is the final stage described in Fe deprivation (Underwood and Suttle, 1999). This stage is characterized by clinical symptoms such as a decrease in growth and feed intake, lethargy, and labored breathing or "thumps". These signs are preceded by and largely caused by the development of hypochromic microcytic anemia (Underwood and Suttle, 1999).

In this study, dietary Fe concentration did not decrease growth performance. Amine et al. (1972a) suggested that BW gain is not a sensitive indicator of Fe deficiency because a decrease in growth is part of the final stage of Fe disorder and occurs only when animals are severely anemic. Studies reported by Hedges and Kornegay (1973), Dove and Haydon (1991), and Yu et al. (2000) also showed no effect on growth performance due to reduced dietary Fe concentrations. The life of the red blood cell in pigs is

approximately 72 d (Withrow and Bell, 1969). Because this study lasted 35 d, the animals did not have complete red blood cell turnover. If the study had been extended another 35 d, pigs fed low-Fe diets might have developed hypochromic microcytic anemia because of inadequate erythrocyte generation, leading to a decrease in growth performance. Talbot and Swenson reported (1970) a decrease in BW and erythrocyte volume per kg of BW after 6 wk in pigs not receiving an Fe supplement.

Pharmacological concentrations of Zn are routinely supplemented to nursery pig diets because of beneficial responses in growth performance (Smith et al., 1997; Hill et al., 2000). Phase 1 and Phase 2 basal diets were formulated to contain pharmacological Zn concentrations, with analyzed concentrations being 1,755 and 1,947 mg Zn/kg, respectively. The basal diet for Phase 3 contained 108 mg Zn/kg. The liver Zn concentration (87.8 mg/kg) of BL pigs in this experiment was similar to hepatic Zn concentration (74 and 105 mg/kg) of pigs weaned at 21 d reported by Hill et al. (1983b), but higher than the hepatic Zn concentration (28 mg/kg) of pigs weaned at 24 d reported by Carlson et al. (1999). Carlson et al. (1999) also reported that liver Zn concentration (218 mg/kg or less) was reflective of the duration (28 d) that pharmacological Zn (3,000 mg/kg) was fed. Following completion of Phase 2 in this experiment, pigs that had consumed diets containing pharmacological Zn concentrations may have had a liver Zn concentration comparable to that reported by Carlson et al. (1999). However, after feeding diets containing 108 mg Zn/kg (adequate) during Phase 3 (14 d), pigs had a hepatic Zn concentration ranging from 42.8 to 56.1 mg/kg. Perhaps during Phase 3, homeostatic mechanisms decreased the hepatic Zn stores accumulated during pharmacological Zn supplementation in Phases 1 and 2.

The roles of Cu in Fe metabolism are many and varied. Ceruloplasmin, a key Cu-containing enzyme, is required for the binding of Fe to Tf via its ferroxidase activity (Osaki and Johnson, 1969). The accumulation of Cu during fetal development results in the neonate having high hepatic Cu stores (Underwood and Suttle, 1999); even so, liver Cu concentration decreases as a result of normal growth and development of the young pig. In this experiment, pigs fed basal diets containing the recommended NRC (1998) Cu concentration for 35 d had decreased liver Cu stores compared with BL pigs. Hepatic Cu concentrations for BL pigs and pigs fed dietary treatments for 35 d were within the normal range for hepatic Cu reported by Underwood and Suttle (1999) for pigs of that age.

Whole-body mineral concentration data are limited, especially for current genetics in the swine industry. Mineral concentrations (Fe, Zn, Cu, Mg, Ca, and P) of BL pigs in this experiment were greater than the mineral concentrations of pigs weaned at 21 d reported by Spray and Widdowson (1950). Also, pigs fed for 35 d in our study had greater Ca, P, and Mg concentrations compared with mineral concentrations reported by Rymarz et al. (1982) and Hendriks and Moughan (1993) for 28.3- and 25.7-kg pigs, respectively. Calcium and P are essential for proper development and maintenance of the skeletal system. Pigs fed for 35 d in the current study had a Ca:P ratio in the body of 1.67, which is slightly higher than the ratios of 1.55, 1.60, and 1.52 reported by Spray and Widdowson (1950), Nielsen (1972), and Hendriks and Moughan (1993), respectively. Spray and Widdowson (1950) also compared nursing pigs receiving a daily dose of supplemental Fe (11 mg/kg BW) during the first 3-wk of life with pigs receiving no supplemental Fe and noted that supplemental Fe greatly increased the amount of Fe in the

body. These results are in agreement with the increases in whole-body Fe concentration due to increases in dietary Fe concentration reported in the current study.

Results obtained for whole-body chemical composition are comparable to those reported by de Lange et al. (2001) for both BL pigs and pigs at the conclusion of the 35-d feeding study. Dietary change from milk to nursery diets is characterized by a change from a liquid diet that is high in fat, to a complex, nutrient-dense pelleted feed. During the initial transition period, the pig is challenged to consume enough feed to meet its energy requirement. Consequently, pigs will mobilize their body lipid reserves to meet the high energy demands during the initial growth phase of the nursery, explaining the observed decrease in percentage of lipid and increase in percentage of water from 19 to 54 d of age. Whole-body protein (15.8 to 16.1%) of pigs analyzed in this study was greater than protein content (13.1%) reported by Rymarz et al. (1982) but comparable to protein content (15.6%) reported by Hendriks and Moughan (1993). Early work by Spray and Widdowson (1950) reported that pigs weaned at 21 d had 13.9% or less protein in contrast to 15.8% protein for BL pigs (19 d of age) reported in the current study. The difference in the percentage of protein between early experiments and this study reflects changes in current genetic selection with an emphasis on increased muscling and growth in the swine industry.

In conclusion, most of the postweaning Fe requirement is thought to be met by the Fe provided by common feed ingredients. Examples of feed ingredients that have a high Fe concentration include dicalcium phosphate, limestone, and blood meal (NRC, 1998). However, the bioavailability of Fe from different sources varies greatly (Kornegay, 1972; Deming and Czarnecki-Maulden, 1989a) and is influenced by such factors as Fe status of

the animal, dietary Fe concentration, and various nutritional and nonnutritional elements within the diet. Consequently, the feed ingredients used in this experiment provided minimal Fe to the basal diets. Even though Fe contributed by feed ingredients provided basal dietary Fe concentrations in excess of the NRC (1998) postweaning requirement (80 mg/kg), the dietary Fe was not adequate to sustain Fe stores in pigs fed lower supplemental Fe concentrations. The decrease in liver Fe stores resulted in a decrease in commonly measurable indicators of Fe status (i.e., Hb, Hct, and PFe). Moreover, the increase in plasma Tf concentration due to lower dietary treatments indicated mobilization of Fe from storage tissue to meet the erythropoietic demands of growing pigs. The increase in whole-body percentage of protein and mineral composition in growing pigs in this experiment compared with pigs in earlier research suggests that erythropoietic demands of growing pigs in this experiment would be greater than those of pigs used in early experiments to derive the postweaning dietary Fe requirement (Pickett et al., 1960). Consequently, the supplementation of 100 mg of Fe/kg of diet, via the highly available ferrous sulfate, was required in addition to the Fe provided by dietary Fe ingredients to alleviate severe decreases in Fe stores.

Implications

Iron contributed by feed ingredients commonly included in nursery pig diets provided analyzed dietary iron concentrations in excess of the postweaning requirement (80 mg per kg), but the iron in these feedstuffs was not sufficient to maintain liver stores of growing pigs. This decrease in liver iron stores decreased indices of iron status that could result in depressed growth. The addition of at least 100 mg of iron per kg of diet via the highly available iron source, ferrous sulfate, is necessary to prevent an excessive decline in iron

stores. This highly adequate and available iron source is especially required by pigs that have been selected for increased growth and muscling, which is typical of pigs currently used in the swine industry.

CHAPTER THREE

EFFECTS OF IRON SUPPLEMENTATION ON BINDING ACTIVITY OF IRON REGULATORY PROTEINS AND THE SUBSEQUENT IMPACT ON GROWTH PERFORMANCE AND INDICES OF HEMATOLOGICAL AND MINERAL STATUS OF YOUNG PIGS

Abstract: Two experiments were conducted to evaluate the effects of supplemental Fe on binding activity of iron regulatory proteins (IRPs) and subsequent impact on growth performance and indices of hematological and mineral status of young pigs. In Exp. 1, littermate male pigs (n = 10; 1.8 kg; 14 ± 1 h) were allotted by BW to two treatments (5 pigs/treatment). Treatments were administered by i.m. injection: 1) Sal (1 mL of sterile saline solution); 2) Fe (1 mL of 200 mg Fe as Fe-dextran). Pigs were bled (d 0 and 13) to determine hemoglobin (Hb), hematocrit (Hct), transferrin (Tf), and plasma Fe (PFe), then killed (d 13) to determine spontaneous and 2-mercaptoethanol (2-ME) inducible IRP binding activity in liver, and liver and whole-body mineral concentrations. Contemporary pigs (n = 5; 2.2 kg; 14 ± 2 h) were killed at d 0 to establish baseline (BL1) measurements. In Exp. 2, pigs (6 pigs/treatment; 6.5 kg; 19 ± 3 d) were fed a basal diet (Phase 1: d 0 to 7; Phase 2: d 7 to 21; Phase 3: d 21 to 35) supplemented with 0 or 150 mg of Fe/kg of diet as ferrous sulfate and killed at d 35 (18.3 kg; 54 ± 3 d). Also, BL2 pigs (n = 5; 5.9 kg; 19 ± 3 d) were killed at the start of Exp. 2 and liver samples were collected and analyzed for IRP binding activity. In Exp. 1, no difference ($P = 0.482$) was observed in ADG between Sal vs. Fe treated pigs. On d 13, Fe treated pigs had greater ($P = 0.001$) Hb, Hct, and PFe and less ($P = 0.002$) Tf than Sal treated pigs. Whole-body Fe concentration was greater ($P = 0.002$) in Fe vs. Sal treated pigs. Treated pigs (Fe or Sal)

had greater ($P = 0.006$) whole-body Cu and less ($P = 0.002$) whole-body Ca, Mg, Mn, P, and Zn concentrations than BL1. Liver Fe concentration was greater ($P = 0.001$) in Fe vs. Sal treated pigs, while liver Fe concentration of Sal treated pigs was less ($P = 0.004$) than BL1 pigs. Sal treated pigs had greater ($P = 0.004$) spontaneous IRP binding activity compared with Fe treated pigs. In Exp. 2, spontaneous ($P = 0.013$) and 2-ME inducible ($P = 0.005$) IRP binding activities were greater in pigs fed diets containing 0 vs. 150 mg of added Fe/kg of diet. No differences ($P = 0.230$) in spontaneous IRP binding activity were observed between BL2 pigs and pigs fed for 35 d. Results suggest that IRP binding activity is reduced by Fe supplementation. Subsequently, other indicators of Fe status are affected via IRP's role in post-transcriptional expression of Fe storage and transport proteins.

Key Words: Iron regulatory protein, Pig, Growth, Whole-body

Introduction

The use of an exogenous source of Fe to prevent Fe deficiency in young pigs has been well documented (Ullrey et al., 1959; Kernkamp et al., 1962; Pollmann et al., 1983) and is standard practice in the swine industry. Utilization of this exogenous Fe to maintain Fe homeostasis involves transferrin and ferritin, the primary transport and storage proteins of Fe, respectively. Iron regulatory proteins (IRPs) control the post-transcriptional expression of these proteins required for the uptake, storage and use of Fe (Eisenstein, 2000). Binding activity of IRPs in rats is responsive to dietary Fe (Chen et al., 1997; Chen et al., 1998). However, IRP binding activity has not been investigated in pigs.

Located within the cytoplasm of cells, IRPs bind to a highly conserved, 28-bp nucleotide sequence known as an iron responsive element (IRE) sequence (Gray et al., 1996). Examples of proteins whose transcribed-messenger (mRNA) contain an IRE include erythroid aminolevulinate synthase (eALAS), transferrin receptor (TfR), and H- and L-ferritin (Eisenstein, 2000). Depending upon the location of the IRE, either the 5'- or 3'-untranslated region (UTR) of the mRNA, the binding of an IRP to an IRE inhibits or stabilizes translation of the mRNA, respectively. The binding activity of IRPs is influenced by Fe status. Iron deficiency causes an increase in binding activity; whereas, Fe sufficiency decreases binding activity (Zahringer et al., 1976; Cox and Adrian, 1993).

The research objectives were to evaluate the effects of supplemental Fe on binding activity of IRPs and the subsequent impact on growth performance and indices of hematological and mineral status of young pigs. Experiment 1 compared neonatal pigs administered Fe-dextran vs. saline, while Exp. 2 evaluated increasing concentrations of supplemental dietary Fe as ferrous sulfate in nursery pigs.

Materials and Methods

Animal Use and Care

These experiments were conducted at the Michigan State University Swine Teaching and Research Facility. Use of animals in these experiments was approved by the All-University Committee on Animal Use and Care (Animal Use Form No. 12/02-164-00).

Experiment 1

Animals, Treatments, and Housing. Ten littermate males (Duroc x Landrace – Yorkshire) were used during a 13-d experiment. Following parturition, pigs were allowed to nurse the sow for 13 to 15 h and then randomly allotted on the basis of initial BW (mean = 1.8 kg) to two treatments in a completely randomized design. Treatments were administered via i.m. injection and were: 1) Sal (1 mL of 0.9% sterile saline solution; Physiological Saline Solution, Phoenix Pharmaceutical Inc., St. Joseph, MO); and 2) Fe (1 mL of 200 mg Fe as Fe-dextran; Duravet Iron Dextran-200, Phoenix Pharmaceutical Inc., St Joseph, MO). There were five pigs per treatment. Following administration of the experimental treatment, pigs were returned to the sow's pen and remained there until completion of the experiment. To prevent blood loss during the experiment, pigs were not castrated, tail docked, or ear notched. During the 13-d experiment, the sow was managed according to facility standard operating procedures. A colostrum sample was collected approximately 5 h after parturition and then milk samples were collected periodically during the 13-d experiment to determine Fe concentration.

Pigs were housed in a temperature-controlled room and grouped in a farrowing pen which provided 3.25 m^2 of total space. Within the farrowing pen, the sow was located in

a hardened steel rod farrowing stall (Delphi Products Company, Delphi, IN) that occupied 1.24 m^2 of space. Pens were located on hardened steel rod flooring (Nooyen Manufacturing Inc., Mt. Sterling, KY). Room temperature was maintained at 19 to 21°C, while zone heating (0.45 m^2 of space) was provided to neonatal pigs by an 85-W heat pad (Osborne Industries Inc., Osborne, KS) located on the floor on one side of the farrowing pen. Exogenous Fe contamination from environmental surroundings was avoided insofar as possible.

Performance, Blood, Tissue, and Whole-body Collection. Pigs were weighed 13 to 15 h post-farrowing (d 0) and then at the conclusion of the 13-d experiment and used in the determination of ADG. Pigs were bled on d 0 and 13 by jugular venipuncture. Initial blood samples were collected using a 5-mL glass syringe (181 USP units of sodium heparin per syringe) with a 22 gauge, 2.54-cm needle. Prior to blood collection, glass syringes were soaked in 30% nitric acid for 6 h, rinsed five times with double-deionized water, and allowed to air-dry. Blood samples on d 13 were drawn by jugular venipuncture into a 10-mL heparinized (143 USP units of sodium heparin per tube) Vacutainer (Becton Dickinson, Franklin Lakes, NJ) tube with a 21 gauge, 3.81-cm needle. For both collections, an aliquot of whole blood was transferred into a polypropylene tube and stored on ice until hemoglobin (Hb) and hematocrit (Hct) analysis could be performed later that day. The remaining blood was centrifuged at 2,000 x g, 4°C, for 10 min (Beckman GS-6KR, Palo Alto, CA). Plasma was collected into polypropylene tubes and stored at -80°C until plasma Fe (PFe) and transferrin (Tf) analysis.

At the conclusion of the experiment, pigs (mean BW = 4.7 kg; 13 d) were killed via cardiac injection of sodium pentobarbital (87 mg/kg of BW). Liver samples were excised and stored (-80°C) in Whirl-Pak bags (Nasco, Fort Atkinson, WI) until being analyzed for minerals. A second liver aliquot was flash-frozen in liquid nitrogen for the determination of IRP-RNA binding activity. The remaining whole-body of each pig was frozen and ground (4732 – Stainless Steel Meat Chopper, Hobart Corp., Troy, OH) three times through a 12.7-mm aperture plate, mixed, and subsampled. Subsamples were freeze-dried (Tri-Philizer MP, FTS Systems Inc., Stone Ridge, NY) and further processed to reduce particle size by submersion in liquid nitrogen and blending in a 1.5 L stainless steel blender (Waring Products Co., New Hartford, CT). Dried, ground subsamples were then stored in Whirl-Pak bags until being analyzed for minerals and chemical composition. Additionally, five littermate pigs of the same farrowing group and similar genetics (Duroc x Landrace – Yorkshire; mean BW = 2.2 kg; 14 ± 1 h) were killed to establish baseline (BL1) measurements for mineral concentrations, IRP binding activity, and chemical composition. Liver and whole-body samples were collected in a similar manner as that previously described.

Mineral, Blood, and Whole-Body Chemical Composition Analysis. Liver, whole-body, and milk samples were analyzed for minerals (Ca, Cu, Fe, Mg, Mn, P, and Zn) as previously described by Rincker et al. (2004). Plasma samples were analyzed for Fe via graphite furnace atomic absorption spectrophotometry (GF90 plus, Thermo Elemental Corp., Franklin, MA). Analysis of whole-body (protein, lipid, and ash) and blood (Hb and Hct) samples was also performed by methods described earlier (Rincker et al., 2004).

All analyses were performed in duplicate. Liver mineral concentrations were reported on a wet basis; whereas, whole-body mineral concentrations were reported on a DM basis.

Preparation and Analysis of IRP Binding Activity. Flash-frozen liver samples were minced with a sterile scalpel and homogenized in 4 volumes of HDGC buffer [20 mmol/L HEPES pH 7.4, 1 mmol/L dithiothreitol, 10% (vol/vol) glycerol, and 2 mmol/L trisodium citrate, 40 mg/L leupeptin, 0.4 mg/L pepstatin, 34.8 mg/L phenylmethylsulfonylfluoride, 4.8 mg/L MG132, and 100 mg/L soybean trypsin inhibitor) using a 15-mL Potter-Elvehjem homogenizer (Tight Pestle A, Wheaton Science Products, Millville, NJ). Liver cytosol was obtained by differential centrifugation. First, the homogenate was centrifuged at 10,000 x g, 4°C, for 15 min (Sorvall Superspeed RC2-B, Kendro Laboratory Products, Asheville, NC). The supernatant was then transferred to a new tube and spun at 100,000 x g, 4°C, for 1 h (Beckman L-80 Ultracentrifuge, Palo Alto, CA) to obtain the liver cytosol. Protein concentration of the liver cytosol was determined by the Bradford Reagent (Sigma, B-6916) assay using BSA (Sigma, A-2153) as a standard (Sigma-Aldrich Co., St. Louis, MO).

Protein-RNA binding activity was performed by gel shift analysis using [^{32}P]RNA of the first 73 nucleotides of the rat L-ferritin 5'UTR (Schalinske and Eisenstein, 1996). Briefly, cell extracts to be analyzed for IRE binding activity were incubated with saturating levels of [^{32}P]RNA (1 nmol/L) in the presence of a final concentration of 5% glycerol, 1 mmol/L magnesium acetate, 20 mmol/L HEPES pH 7.5, 75 mmol/L potassium chloride, and 20 mg/L nuclease free BSA in a final volume of 30 μL. Binding reactions were started by the addition of [^{32}P]RNA and were performed at room temperature for 10 min. Then 3 μL of heparin (5 g/L in deionized water) was added and

the sample incubated for another 5 min after which 25 μL of the sample was loaded onto a native 4% polyacrylamide (60:1 acrylamide/bisacrylamide) gel in 0.5X Tris-borate-EDTA buffer (Barton et al., 1990). The samples were electrophoresed at 160 V for 50 min. The optimal amount of protein used for the gel shift assay was 10 μg of cytosolic protein. The amount of IRP present in active and inactive (i.e., cytosolic-aconitase) forms was assessed using 4% 2-mercaptoethanol (2-ME) (Klausner et al., 1993). In the presence of 2-ME, 2.5 μg of cytosolic protein was the optimal amount for the gel shift assay. Iron regulatory protein-RNA binding activity was quantified by liquid scintillation counting (Beckman LS 5000TD) as described by Schalinske and Eisenstein (1996). Results are expressed as pmol of [^{32}P]RNA bound per mg of cytosolic protein. Additional details of the gel shift assay have been described previously (Barton et al., 1990).

Gel Electrophoresis and Western Blot Analysis. Tissue IRP presence was determined by SDS-PAGE followed by Western blot analysis using a rabbit polyclonal antibody raised against rat liver IRP. Iron regulatory protein was analyzed using 30 μg liver homogenate protein. After proteins were electrophoretically separated in 8% polyacrylamide gels, they were electrophoretically transferred to polyvinylidene difluoride membranes. Next, membranes were blocked for 2 h at room temperature in PBS solution, pH 7.4 [5.0% (wt/vol) nonfat dry milk, 0.1% (vol/vol) Tween-20, 8.0 g/L sodium chloride, 0.2 g/L potassium chloride, 1.4 g/L dibasic sodium phosphate, and 0.2 g/L monobasic potassium phosphate]. Then, membranes were incubated with the polyclonal antibody (1:5,000 dilution) in PBS solution, followed by washing in PBS solution. Subsequently, membranes were incubated with goat anti-rabbit IgG conjugated

with horseradish peroxidase (1:1,000 dilution) in PBS solution (Sigma-Aldrich Co). Immunoreactive proteins were visualized using the TMB Membrane Peroxidase Substrate System (Kirkegaard & Perry Lab., Gaithersburg, MD).

Experiment 2

Pigs used in Exp. 2 were a subset of a larger data set previously reported (Rincker et al., 2004) and used to determine the effects of dietary Fe supplementation on growth performance, hematological status, and whole-body mineral concentrations of nursery pigs. The subset of pigs consisted of BL2 pigs (n = 5; mean BW = 5.9 kg; 19 ± 3 d), killed at the initiation of the study, and pigs fed the basal diet supplemented with 0 or 150 mg of Fe per kg of diet as ferrous sulfate (6 pigs/treatment; mean BW = 18.3 kg; 54 ± 3 d), killed at the conclusion of the 35-d feeding experiment. Liver samples were collected from these pigs and analyzed for IRP binding activity via the laboratory methods previously described in Exp. 1.

Statistical Analysis

In Exp. 1, data were analyzed as a completely randomized design using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). Pig was the experimental unit. Two preplanned orthogonal comparisons were made: 1) BL1 pigs vs. pigs receiving a Sal or Fe i.m. injection; and 2) Sal vs. Fe i.m. injection. For blood response criteria, d 0 Hb, Hct, PFe, and Tf values were used as a covariate for their respective individual analyses. In Exp. 2, data were analyzed using the MIXED model methodology of SAS. Two preplanned orthogonal comparisons were made: 1) BL2 pigs vs. both Fe treatments (0 and 150 mg of added Fe/kg of diet); and 2) 0 vs. 150 mg of added Fe/kg of diet.

Differences were considered significant at the level of $P < 0.050$ and highly significant at the level of $P < 0.010$.

Results and Discussion

The hepatic Fe stores of a neonatal pig combined with the low Fe concentration in sow's milk are not sufficient to meet the rapid growth and increase in blood volume during this time period. Braude et al. (1962) estimated that a suckling pig must retain 21 mg of Fe/kg of BW gain in order to maintain satisfactory Hb and storage Fe concentrations. In Exp. 1, the mean Fe concentration in sow's milk was 1.02 mg/L. Thus, neonatal pigs can not consume adequate quantities of milk to meet their Fe requirement.

Whole blood Hb concentration and Hct percentage are commonly used as indicators of a pig's Fe status because of their ease of measurement. A whole blood Hb concentration of 100 g/L is considered adequate, while 80 g/L suggests borderline anemia, and 70 g/L or less indicates anemia (Zimmerman, 1980). There were no differences ($P > 0.100$) in Hb, Hct, and PFe at the start of experiment Exp. 1 (Table 1). On d 13, Fe treated pigs had greater ($P = 0.001$) Hct percentage and Hb and PFe concentration compared with Sal treated pigs. The mean Hb concentration of Sal treated pigs (59 g/L) indicates that they were anemic by d 13. Pollmann et al. (1983) reported a similar decrease in Hb concentration and Hct percentage at 10-d of age in pigs receiving no supplemental Fe compared with pigs receiving 200 mg of Fe via an i.m. injection of Fe-dextran at 1-d of age.

Table 1. Effects of Fe administration on neonatal pig growth performance and hematological status in Exp. 1[a]

	Treatment[b]			*P*-value[c]
Item	Sal	Fe	SEM	Sal vs. Fe
ADG, g/d				
d 0 to 13	214.24	245.64	30.09	0.482
Hemoglobin, g/L				
d 0	124.45	116.94	9.50	0.519
d 13	58.61	120.89	3.77	0.001
Hematocrit, %				
d 0	39.56	36.72	1.90	0.321
d 13	22.59	44.69	0.47	0.001
Plasma Fe, mg/L				
d 0	1.61	1.76	0.36	0.785
d 13	0.23	1.72	0.05	0.001
Plasma transferrin, g/L				
d 0	12.13	13.14	1.80	0.703
d 13	65.33	39.21	3.78	0.002

[a]Data are least squares means (n = 5 per treatment; initial mean BW = 4.7 kg; initial age = 14 ± 1 h).
[b]Sal (1 mL of 0.9% sterile saline solution via an i.m. injection; Physiological Saline Solution, Phoenix Pharmaceutical Inc., St. Joseph, MO); Fe (1 mL of 200 mg Fe as Fe-dextran via an i.m. injection; Duravet Iron Dextran-200, Phoenix Pharmaceutical Inc., St Joseph, MO).
[c]Preplanned orthogonal comparison was: 1) Sal vs. Fe i.m. injection.

Examples of proteins whose expression is regulated by IRP binding activity include H- and L-ferritin (Aziz and Munro, 1987), TfR (Casey et al., 1988), and eALAS (May et al., 1990). These proteins are critical in maintaining Fe homeostasis. By determining IRP binding activity in addition to the traditional measurements (i.e. Hb concentration and Hct percentage) used to establish Fe status, a more-sensitive assessment of a young pig's Fe state can be made during this rapid growth period.

A representative immunoblot of IRP1 is shown in Figure 1. The reported molecular weight of rat IRP1 is 98 kDa (Hentze and Argos, 1991). The molecular mass of porcine IRP1 is similar to that of rat. A representative autoradiograph of spontaneous and 2-ME inducible RNA binding activity of cytosolic IRP is shown in Figure 2. In Exp. 1, pigs receiving a Sal injection had greater ($P = 0.004$) spontaneous IRP binding activity than Fe treated pigs (Table 2). This is in agreement with Chen et al. (1997) who reported greater IRP binding activity in weanling rats fed diets containing 2 mg of Fe/kg of diet compared with rats fed adequate Fe diets (37 mg of Fe/kg of diet). Both treatment groups had greater ($P = 0.018$) spontaneous IRP binding activity compared with BL1 pigs. Based on the role of IRPs, the greater spontaneous IRP binding activity noted in Sal treated pigs suggests that the synthesis of proteins involved in Fe storage was inhibited, while the synthesis of proteins involved in Fe transport and uptake were increased to meet erythropoietic demands of tissues compared with Fe treated pigs.

In the presence of 2-ME, inactive IRPs (i.e. cytosolic aconitase) are converted to an active, high-affinity RNA binding form. Thus, the amount of 2-ME inducible RNA binding activity is a measure of the total amount of IRP protein present (Schalinske and Eisenstein, 1996). No difference ($P = 0.237$) in 2-ME inducible IRP binding activity was

observed between injection treatments; however, both treatment groups had less (P = 0.002) 2-ME inducible IRP binding activity than BL1 pigs. This suggests that neonatal pigs are born with a portion of the IRP protein in an inactive form. Then, if pigs are not supplemented with Fe soon after birth to meet the body's demands, inactive IRP protein is converted to an active form and an increase in IRP binding activity occurs, as was observed in Sal treated pigs. Chen et al. (1997) reported that the amount of 2-ME inducible IRP binding activity in rats ingesting diets containing 2 or 11 mg of Fe/kg of diet was greater than that of controls (37 mg of Fe/kg of diet).

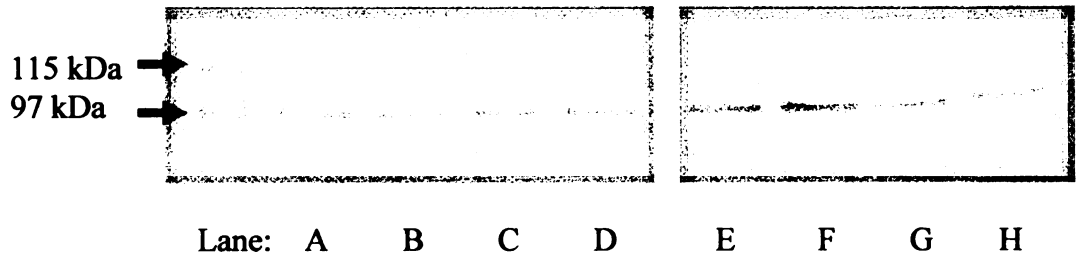


Figure 1. A representative immunoblot of iron regulatory protein 1(IRP1) is shown. In Exp. 1, treatments are: BL1 (n = 5; mean BW = 2.2 kg; 14 ± 1 h); Sal (n = 5; mean BW = 4.7 kg; 13 d; 1 mL of 0.9% sterile saline solution via an i.m. injection; Physiological Saline Solution, Phoenix Pharmaceutical Inc., St. Joseph, MO); Fe (n = 5; mean BW = 4.7 kg; 13 d; 1 mL of 200 mg Fe as Fe-dextran via an i.m. injection; Duravet Iron Dextran-200, Phoenix Pharmaceutical Inc., St Joseph, MO). In Exp. 2, treatments are: BL2 (n = 5; mean BW = 5.9 kg; 19 ± 3 d); for 0 and 150 mg of added Fe/kg of diet (n = 6 per treatment; mean BW = 18.3 kg; 54 ± 3 d). Lane A represents the pre-stained molecular weight markers, β-galactosidase and bovine serum albumin, which have a weight of 115 and 97 kDa, respectively. Lanes B through D represent IRP1 in Sal, Fe, and BL1 pigs in Exp. 1, respectively; whereas, lanes E through G represent IRP1in 0, 150, and BL2 pigs in Exp. 2, respectively. Lane H represents IRP1 in rat (control).

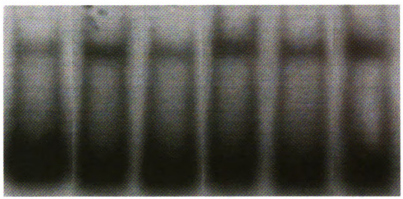


Figure 2. A representative autoradiograph of spontaneous and 2-ME (2-mercaptoethanol) inducible RNA binding activity of cytosolic iron regulatory protein (IRP) is shown. Treatments are: BL1 (n = 5; mean BW = 2.2 kg; 14 ± 1 h); Sal (n = 5; mean BW = 4.7 kg; 13 d; 1 mL of 0.9% sterile saline solution via an i.m. injection; Physiological Saline Solution, Phoenix Pharmaceutical Inc., St. Joseph, MO); Fe (n = 5; mean BW = 4.7 kg; 13 d; 1 mL of 200 mg Fe as Fe-dextran via an i.m. injection; Duravet Iron Dextran-200, Phoenix Pharmaceutical Inc., St Joseph, MO). Lanes A through C represent spontaneous IRP binding activity in BL1, Sal, and Fe pigs, respectively; whereas, lanes D through F represent 2-mercaptoethanol inducible IRP binding activity in BL1, Sal, and Fe pigs, respectively. The optimal amount of protein used for the gel shift assay when determining spontaneous IRP binding activity was 10 μg of cytosolic protein. In the presence of 4% 2-ME, 2.5 μg of cytosolic protein was the optimal amount for the gel shift assay.

Table 2. Effects of Fe administration on young pig liver iron regulatory protein (IRP) binding activity in Exp. 1 and Exp. 2

	Exp. 1[a]						Exp. 2[b]					
		Treatment[c]			*P*-value[d]			Supplemental Fe[e]			*P*-value[f]	
IRP binding activity[g]	BL1	Sal	Fe	SEM	BL1 vs. Sal & Fe	Sal vs. Fe	BL2	0	150	SEM	BL2 vs. Fe	0 vs. 150
Spontaneous	0.165	0.272	0.181	0.018	0.018	0.004	0.236	0.241	0.178	0.017	0.230	0.013
2-ME inducible[h]	1.245	0.988	0.874	0.075	0.002	0.237	0.556	0.803	0.673	0.030	0.001	0.005

[a]Data are least squares means (BL1: n = 5; mean BW = 2.2 kg; 14 ± 1 h; for Sal and Fe n = 5 per treatment; mean BW = 4.7 kg; 13 d).

[b]Data are least squares means (BL2: n = 5; mean BW = 5.9 kg; 19 ± 3 d; for 0 and 150 n = 6 per treatment; mean BW = 18.3 kg; 54 ± 3 d).

[c]Sal (1 mL of 0.9% sterile saline solution via an i.m. injection; Physiological Saline Solution, Phoenix Pharmaceutical Inc., St. Joseph, MO); Fe (1 mL of 200 mg Fe as Fe-dextran via an i.m. injection; Duravet Iron Dextran-200, Phoenix Pharmaceutical Inc., St Joseph, MO).

[d]Preplanned orthogonal comparisons were: 1) BL1 pigs vs. pigs receiving either a Sal or Fe i.m. injection; 2) Sal vs. Fe i.m. injection.

[e]Basal diet supplemented with 0 or 150 mg of Fe per kg of diet.

[f]Preplanned orthogonal comparisons were: 1) BL2 pigs vs. both Fe treatments (0 and 150 mg added Fe/kg of diet); 2) 0 vs. 150 mg added Fe/kg of diet.

[g]Results are expressed as pmol of [^{32}P]RNA bound per mg of cytosolic protein.

[h]2-ME inducible IRP is the amount of RNA binding activity determined in the presence of 4% 2-ME (2-mercaptoethanol).

During the initial stage of Fe depletion, liver, kidney, and spleen Fe stores (i.e., ferritin and hemosiderin) are reduced (Underwood and Suttle, 1999). Liver Fe concentration (Table 3) of BL1 pigs was considered adequate for neonatal pigs as reported by Underwood and Suttle (1999). Liver Fe concentrations of Sal treated pigs was less (P = 0.004) than BL1 pigs; whereas, Fe treated pigs had a greater (P = 0.004) liver Fe concentration in contrast to BL1 pigs. Iron treated pigs had a 25-fold greater (P = 0.001) liver Fe concentration than Sal treated pigs. Similar results were reported by Pollmann et al. (1983) who noted a 15-fold increase in nonheme liver Fe at 3-wk of age in pigs receiving 200 mg of Fe via an i.m. Fe-dextran injection at 1-d of age compared with pigs receiving no supplemental Fe. As previously mentioned, Sal treated pigs had increased IRP binding activity. The increase in binding activity of IRPs results in IRPs binding to IREs in the mRNA and inhibition of ferritin synthesis. Chen et al. (1997) reported that a biphasic relationship existed between IRP binding activity and ferritin concentration in rat liver. They suggested that at a critical concentration of Fe, ferritin synthesis is either rapidly and fully activated or repressed depending on the direction of change in Fe concentration.

Table 3. Effects of Fe administration on neonatal pig liver mineral concentrations (wet basis), whole-body mineral concentrations (DM basis), and chemical composition in Exp. 1[a]

Item	BL1	Treatment[b]		SEM	*P*-value[c]	
		Sal	Fe		BL1 vs. Sal & Fe	Sal vs. Fe
Liver minerals						
DM, %	25.77	25.71	25.93			
Fe, mg/kg	31.32	16.03	408.03	41.34	0.004	0.001
Cu, mg/kg	37.11	60.27	46.29	6.07	0.050	0.129
Zn, mg/kg	15.00	56.19	53.42	1.68	0.001	0.287
Mg, mg/kg	180.70	195.77	195.83	5.26	0.037	0.993
Mn, mg/kg	1.82	1.72	2.66	0.17	0.100	0.002
Ca, mg/kg	28.34	39.34	43.06	1.98	0.001	0.207
P, g/kg	1.97	2.89	2.95	0.06	0.001	0.464
Whole-body minerals						
Fe, mg/kg	124.73	61.33	160.18	17.23	0.521	0.002
Cu, mg/kg	7.61	9.58	9.36	0.45	0.006	0.731
Zn, mg/kg	78.80	66.93	62.84	2.90	0.002	0.337
Mg, mg/kg	1,278.88	911.07	891.33	34.68	0.001	0.694
Mn, mg/kg	2.07	1.14	1.04	0.12	0.001	0.548
Ca, g/kg	59.96	26.53	25.07	2.01	0.001	0.619
P, g/kg	28.79	16.53	16.06	0.84	0.001	0.697
Whole-body chemical composition						
Water, %	80.72	70.16	69.25	0.79	0.001	0.428
Protein, %	12.73	14.11	14.29	0.21	0.001	0.556
Lipid, %	2.81	12.98	13.70	0.82	0.001	0.543
Ash, %	3.75	2.75	2.76	0.11	0.001	0.960

[a]Data are least squares means (BL1: n = 5; mean BW = 2.2 kg; 14 ± 1 h; for other data n = 5 per treatment; mean BW = 4.7 kg; 13 d).

[b]Sal (1 mL of 0.9% sterile saline solution via an i.m. injection; Physiological Saline Solution, Phoenix Pharmaceutical Inc., St. Joseph, MO); Fe (1 mL of 200 mg Fe as Fe-dextran via an i.m. injection; Duravet Iron Dextran-200, Phoenix Pharmaceutical Inc., St Joseph, MO).

[c]Preplanned orthogonal comparisons were: 1) BL1 pigs vs. pigs receiving either a Sal or Fe i.m. injection; 2) Sal vs. Fe i.m. injection.

Transferrin is the primary means of interorgan transport of nonheme Fe. The abundance of plasma Tf is inversely related to Fe status in rats (Morton and Tavill, 1977; Zakin, 1992) and chicks (McKnight et al., 1980). On d 13, Sal treated pigs had greater (P = 0.002) plasma Tf concentration than Fe treated pigs (Table 1). We previously reported (Rincker et al., 2004) a linear decrease in plasma Tf concentration in pigs fed increasing dietary Fe concentrations (0, 25, 50, 100, and 150 mg of added Fe/kg). Results from the present experiment suggest that Sal treated pigs had a greater need to transport nonheme Fe to Fe-dependent tissues. The uptake of nonheme Fe by tissue is a coordinated process between Tf and TfR. The mRNA encoding TfR contains IREs that IRPs bind to and stabilize for translation of the protein. Thus, the increased IRP binding activity observed in Sal treated pigs suggests that TfR synthesis was increased, along with the noted increase in plasma Tf concentration, to meet the demands of Fe-dependent tissue. Cox and Adrian (1993) reported that human Tf synthesis is regulated by Fe post-transcriptionally and that similarities may exist between the mechanisms (i.e. IRP binding activity) regulating Tf and ferritin synthesis; however, this has not been studied in the pig.

No visual symptoms of Fe deficiency (i.e. labored breathing, rough hair coat, wrinkly skin, or listlessness) were observed among the experimental pigs. Amine et al. (1972a) suggested that BW gain is not a sensitive indicator of Fe adequacy because a decrease in growth is one of the last signs of an Fe disorder following the development of hypochromic-microcytic anemia. For the 13-d experiment, no difference (P = 0.482) was observed in ADG between pigs receiving a Sal or Fe injection (Table 1). Talbot and Swenson (1970) reported no difference in BW at 14-d of age between pigs receiving an

i.m. injection of 0.85% NaCl solution or 150 mg of Fe via an i.m. Fe-dextran injection at 1-d of age. Ullrey et al. (1959) did not observe a decrease in BW until 5-wk of age in pigs receiving no supplemental Fe compared with pigs receiving a 100 mg i.m. injection of ferrous Fe at 3- or 4-d of age. Similar results were reported by Kernkamp et al. (1962) who compared the growth of pigs receiving various Fe sources, including ferric ammonium citrate, Fe-dextran, and Fe-dextrin at 3-d of age with pigs receiving no supplemental Fe. These authors did not observe a decrease in growth after 14 d; however, pigs receiving no supplemental Fe had lower BW gain after 28 d in contrast to pigs receiving any of the other Fe sources. In pigs, the life of the red blood cell is approximately 72 d (Withrow and Bell, 1969). Because this study lasted 13 d, the animals did not have complete red blood cell turnover. A decrease in growth performance would most likely have occurred if Exp. 1 had been extended for a longer duration.

Pigs 13-d of age had greater liver Cu ($P = 0.050$), Zn ($P = 0.001$), Mg ($P = 0.037$), Ca ($P = 0.001$), and P ($P = 0.001$) concentrations compared with BL1 pigs (Table 3). Sow milk nutrient composition changes with stage of lactation. Hill et al. (1983a) reported that micro-mineral concentrations (Cu, Fe, and Zn) are greater in colostrum and then decrease as lactation progresses; whereas, macro-mineral concentrations (Ca, Mg, and P) increase during the progression of lactation. Pigs receiving an Fe injection had a greater ($P = 0.002$) liver Mn concentration than Sal treated pigs. Hill and Matrone (1970) reported than an interrelationship exists between Fe and Mn because of their similar chemical and physical properties (i.e. similar electronic structure). In contrast to our

results, a previous report indicates that Mn absorption by the proximal intestine is increased during Fe deficiency in rats (Thomson et al., 1971).

Shown in Table 3 are whole-body mineral concentrations of neonatal pigs. Pigs in the Fe treatment had a 2.5-fold greater ($P = 0.002$) whole-body Fe concentration compared with Sal treated pigs. No differences ($P > 0.100$) were observed in Cu, Zn, Mg, Mn, Ca, and P whole-body concentration between pigs receiving a Sal or Fe injection at 1-d of age. However, pigs 13-d of age had a greater whole-body Cu ($P = 0.006$) and less whole-body Zn ($P = 0.002$), Mg ($P = 0.001$), Mn ($P = 0.001$), Ca ($P = 0.001$), and P ($P = 0.001$) concentrations than BL1 pigs. In contrast, Mahan and Shields (1998) reported that Ca, Mg, Mn, P, and Zn whole-body concentrations (expressed on a fat-free, empty body weight basis) increased during the nursing period because of the transfer of minerals from maternal tissue stores to the tissue of nursing pigs via milk consumption. If we had accounted for the percentage lipid in whole-body samples when expressing our results, then an increase from d 0 to 13 in whole-body mineral concentrations (Ca, Mg, Mn, P, and Zn) would have been noted.

Whole-body chemical composition of neonatal pigs in Exp. 1 is also presented in Table 3. Baseline pigs had a greater percentage water and ash and a smaller percentage protein and lipid ($P = 0.001$) compared with 13-d old pigs. The increased percentage lipid in pigs 13-d of age is the result of the consumption of sow's milk that contains 30 to 40% fat on a DM basis (deMann and Bowland, 1963). Given the close associations among body protein, water, and ash, most of the variation in chemical body composition between different groups of pigs can be attributed to variation in body lipid content (Hendriks and Moughan, 1993; Bikker et al., 1996a; Bikker et al., 1996b). Thus, the 4.5-

fold increase in the percentage lipid in pigs 13-d of age compared with BL1 pigs is the reason the percentage water and ash decreased from d 0 to 13. No differences ($P >$ 0.100) were observed in whole-body percentage water, protein, lipid, and ash between Fe vs. Sal treated pigs.

In Exp. 2, spontaneous IRP binding activity was greater ($P = 0.013$) in pigs fed diets containing 0 vs. 150 mg of added Fe/kg of diet. These results further support those we previously published in which Hct percentage and Hb, PFe, and liver Fe concentrations were less and plasma Tf concentration was greater in pigs fed diets supplemented with 0 vs. 150 mg of Fe/kg of diet (Rincker et al., 2004). This suggests that the dietary Fe was inadequate to meet the Fe requirement of pigs fed 0 mg of added Fe/kg of diet. Thus, IRP binding activity was increased in pigs fed diets supplemented with 0 mg of Fe/kg of diet and the synthesis of Fe storage proteins was inhibited, while the synthesis of proteins involved in Fe transport and uptake was increased to meet the erythropoietic tissue demands.

No difference ($P = 0.230$) in spontaneous IRP binding activity was observed between BL2 pigs and pigs fed the experimental diets for 35 d. We previously reported (Rincker et al., 2004) the plasma Tf and liver Fe concentrations for BL2 pigs (52.20 g/L and 247 mg/kg, respectively) and pigs fed diets containing 0 mg of added Fe/kg of diet (44.66 g/L and 35 mg/kg, respectively). The high liver Fe concentration in BL2 pigs is indicative of the Fe stores that were accumulated following administration of an i.m injection of Fe-dextran at 1 to 2 d of age. However, the high IRP binding activity and plasma Tf concentration suggests that BL2 pigs were drawing upon their Fe stores to meet the rapid growth demands and increase in blood volume at this age.

Not only was 2-ME inducible IRP binding activity greater ($P = 0.005$) in pigs fed diets containing 0 vs. 150 mg of added Fe/kg of diet, but it was also greater ($P = 0.001$) in both groups fed dietary treatments for 35 d in contrast to BL2 pigs. Chen et al. (1997) also reported an increase in the amount of 2-ME inducible RNA binding activity in Fe deficient rats. They suggested that more of the newly synthesized binding protein is diverted to the high affinity RNA binding form in Fe deficient animals.

Based upon the traditional measurements of Fe status (i.e. Hb concentration and Hct percentage), Fe deficiency was induced in Sal injected pigs. The increased IRP binding activity suggests that Sal treated pigs were not storing Fe, but instead were transporting Fe to meet the erythropoietic demands of tissues. This is evident by the decreased liver Fe and increased plasma Tf concentrations observed in these pigs. Results of Exp. 2 suggest that the dietary Fe was inadequate to meet the Fe requirement of pigs fed 0 mg of added Fe/kg of diet. Thus, IRP binding activity was increased in these pigs, which would be expected to decrease the synthesis of Fe storage proteins and increase the synthesis of proteins involved in Fe transport and uptake. This is supported by the lower liver Fe and greater plasma Tf concentrations of pigs fed diets containing 0 vs. 150 mg of added Fe/kg of diet that we reported earlier (Rincker et al., 2004). In conclusion, Fe supplementation influences IRP binding activity in young pigs. By having post-transcriptional control over proteins involved in Fe transport and storage, IRPs are key regulators of Fe homeostasis in the young pig.

Implications

Our research shows that the use of an exogenous iron source to prevent iron deficiency in neonatal pigs influences the binding activity of iron regulatory proteins.

Based upon their role in modulating the post-transcriptional expression of proteins required for the uptake, storage, and use of iron, an increase in iron regulatory protein binding activity can indicate the initial stage of iron depletion not found by measuring hemoglobin and hematocrit. By determining iron regulatory protein binding activity, hemoglobin concentration, and hematocrit percentage, a better assessment of a young pig's Fe state can be made during this rapid growth period. During the subsequent nursery period, a highly available dietary iron source such as ferrous sulfate is necessary to support rapid growth. As in the neonatal phase, the dietary iron source influences binding activity of iron regulatory proteins and maintains iron homeostasis via the expression of iron transport and storage proteins.

SUMMARY AND CONCLUSIONS

The supplementation of an exogenous Fe source to prevent Fe deficiency in young pigs is standard practice in today's swine industry. This exogenous Fe source influences the binding activity of IRPs that regulate the post-transcriptional expression of proteins required for the uptake, storage, and use of iron. Based upon the traditional measurements of Fe status (i.e. Hb concentration and Hct percentage), Fe deficiency was induced in Sal treated pigs. Iron deficient pigs had greater IRP binding activity that subsequently decreased the synthesis of Fe storage proteins and increased the synthesis of Fe transport proteins to meet the erythropoietic demands of tissues. This is evident by the decreased liver Fe and increased plasma Tf concentrations observed in these pigs. In Exp. 2, results suggest that the dietary Fe was inadequate to meet the Fe requirement of pigs fed 0 mg of added Fe/kg of diet. Thus, IRP binding activity was increased in these pigs, which would be expected to decrease the synthesis of Fe storage proteins and increase the synthesis of proteins involved in Fe transport and uptake. This is supported by the lower liver Fe and greater plasma Tf concentrations of pigs fed diets containing 0 vs. 150 mg of added Fe/kg of diet. The addition of at least 100 mg of Fe/kg of diet via the highly available Fe source, ferrous sulfate, is necessary to prevent an excessive decline in Fe stores. This highly adequate and available iron source is especially required by pigs that have been selected for increased growth and muscling, which is typical of pigs currently used in the swine industry.

APPENDIX

EFFECTS OF DIETARY ZINC AND IRON SUPPLEMENTATION ON MINERAL EXCRETION, BODY COMPOSITION AND MINERAL STATUS OF NURSERY PIGS

ABSTRACT: Two experiments were conducted to evaluate the effects of dietary Zn and Fe supplementation on mineral excretion, body composition, and mineral status of nursery pigs. In Exp. 1 (n = 24; 6.5 kg; 18 ± 2 d) and 2 (n = 24; 7.2 kg; 20 ± 1 d), littermate crossbred barrows were weaned and randomly allotted by BW, within litter, to dietary treatments and housed individually in stainless steel pens. In Exp. 1, Phases 1 (d 0 to 7) and 2 (d 7 to 14) diets were: 1) NC (negative control, no added Zn source); 2) ZnO (NC + 2,000 mg/kg as Zn oxide); 3) ZnM (NC + 2,000 mg/kg as Zn methionine). In Exp. 2, diets for each phase (Phase 1: d 0 to 7; Phase 2: d 7 to 21; Phase 3: d 21 to 35) were the basal diet supplemented with 0, 25, 50, 100, and 150 mg/kg as ferrous sulfate. Orts, feces, and urine were collected daily in Exp. 1; whereas, pigs had a 4-d adjustment period followed by a 3-d collection period (Period 1: d 5 to 7; Period 2: d 12 to 14; Period 3: d 26 to 28) during each phase in Exp. 2. Pigs were bled on d 0, 7, and 14 in Exp. 1 and d 0, 7, 21, and 35 in Exp. 2 to determine hemoglobin (Hb), hematocrit (Hct), and plasma Cu, (PCu), Fe (PFe), and Zn (PZn). Pigs in Exp. 1 were killed at d 14 (mean BW = 8.7 kg) to determine whole-body, liver, and kidney mineral concentrations. There were no differences ($P > 0.100$) in growth performance in Exp. 1 or 2. In Exp. 1, pigs fed ZnO or ZnM diets had greater ($P < 0.001$) dietary Zn intake during the 14 d study and greater fecal Zn excretion during Phase 2 compared with pigs fed the NC diet. Pigs fed 2,000 mg/kg, regardless of Zn source, had greater ($P < 0.010$) PZn on d 7 and 14 than pigs fed

the NC diet. Whole-body Zn, liver Fe and Zn, and kidney Cu concentrations were greater ($P < 0.010$); while kidney Fe and Zn concentrations were less ($P < 0.010$) in pigs fed pharmacological Zn diets than pigs fed the NC diet. In Exp. 2, dietary Fe supplementation tended to increase (linear, $P = 0.075$) dietary DM intake, resulting in a linear increase ($P < 0.050$) in dietary Fe, Mg, Mn, P, and Zn intake. Subsequently, a linear increase ($P < 0.010$) in fecal Fe and Zn excretion was observed. Increasing dietary Fe resulted in a linear increase in Hb, Hct, and PFe on d 21 ($P < 0.050$) and 35 ($P < 0.010$). Results suggest that dietary Zn or Fe additions increase mineral status of nursery pigs. Once tissue mineral stores are loaded, dietary minerals in excess of the body's requirement are excreted.

Key Words: Iron, Nursery pig, Nutrient balance, Whole-body, Zinc

Introduction

Nutrient management plans focus primarily on P and N with little regard to other nutrients. Yet, the accumulation of other minerals in soil can hinder crop production (Takkar and Mann, 1978). Swine manure volume and its nutrient content have been estimated based on standard values reported by ASAE (1988). However, current values representing today's genetics and management practices are needed by producers to estimate the swine manure volume and its nutrient content.

The addition of 2,000 to 3,000 mg of Zn/kg of diet as Zn oxide to nursery pig diets is a common practice in the swine industry because of the reported improvements in growth performance (Smith et al., 1997; Hill et al., 2000). However, feeding pharmacological Zn to nursery pigs can result in a significant increase in the total quantity of Zn excreted during a pig's entire production cycle (Meyer et al., 2002). This is a particular concern in today's swine industry where intensive production units have increased the volume of manure produced on facilities that may have limited access to land for application.

The analyzed Fe concentration of most nursery diets is in excess of the NRC (1998) postweaning dietary Fe requirement, 80 mg/kg. This is because many feed ingredients have a high Fe concentration, including dicalcium phosphate, limestone, and blood meal. However, the availability of Fe from different sources varies greatly (Deming and Czarnecki-Maulden, 1989a). Similar to Zn, environmental concerns exist because of the high dietary Fe concentrations and variability in the Fe availability of feedstuffs.

The research objectives were to determine the effects of dietary Zn and Fe supplementation on mineral excretion, nutrient balance, and mineral status of nursery pigs. Experiment 1 compared an organic (Zn methionine) vs. inorganic (Zn oxide) Zn

form immediately (14 d) postweaning, while Exp. 2 evaluated increasing concentrations of supplemental dietary Fe as ferrous sulfate.

Materials and Methods

Animal Use and Care

These experiments were conducted at the Michigan State University Swine Teaching and Research Facility. Use of animals in these experiments was approved by the All-University Committee on Animal Use and Care (Exp. 1: 12/99-159-00; Exp. 2: 12/02-164-00).

Experiment 1

Animals and Treatments. Eight sets of three littermate barrows (Duroc x Landrace – Yorkshire) were weaned at 18 ± 2 d and individually housed in metabolism pens during a 14-d feeding experiment. Pigs were randomly allotted, within litter, on the basis of initial BW (mean = 6.5 kg) to one of three dietary treatments in a randomized complete block design. There were eight pigs per treatment.

Dietary treatments (Table 1) consisted of: 1) NC (negative control, no added Zn source); 2) ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); 3) ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine). Complexity of the diets changed with phases (Phase 1: d 0 to 7; Phase 2: d 7 to 14) to meet or exceed NRC (1998) nutrient recommendations, excluding Zn, and to satisfy changes in digestive capabilities of the weanling pig. Experimental Zn concentrations were obtained by replacing an appropriate amount of corn with the respective Zn source. In order to equalize the methionine concentration in the diets, supplemental methionine was reduced in the ZnM diet by the amount calculated to be present in the Zn methionine source. Diets were fed in meal form.

Table 1. Composition of basal diets used in Exp. 1 (as-fed basis)[a]

	Phase 1, d 0 to 7			Phase 2, d 7 to 14		
Ingredient, %	NC	ZnO	ZnM	NC	ZnO	ZnM
Corn[b]	38.40	38.10	36.30	48.49	48.18	44.69
Soybean meal (44% CP)	19.10	19.11	19.29	23.21	23.24	23.56
Whey, spray dried	20.00	20.00	20.00	20.00	20.00	20.00
Lactose	10.00	10.00	10.00	-	-	-
Plasma protein	6.00	6.00	6.00	-	-	-
Soy protein concentrate	-	-	-	4.00	4.00	4.00
Soybean oil	3.00	3.00	3.00	1.00	1.00	2.50
L-Lysine HCl	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.10	0.10	-	0.06	0.06	-
Dicalcium phosphate	0.92	0.93	0.95	0.90	0.90	0.94
Limestone	0.95	0.95	0.93	0.78	0.78	0.75
Sodium chloride	0.20	0.20	0.20	0.25	0.25	0.25
Selenium-90	0.08	0.08	0.08	0.06	0.06	0.06
Mineral premix[c]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin premix[d]	0.60	0.60	0.60	0.60	0.60	0.60
Zn Oxide	-	0.28	-	-	0.28	-
Zn Methionine	-	-	2.00	-	-	2.00
Calculated composition						
Lysine, %	1.35	1.35	1.35	1.25	1.25	1.25
Ca, %	0.85	0.85	0.85	0.80	0.80	0.80
P, %	0.65	0.65	0.65	0.60	0.60	0.60
Analyzed composition						
Cu, mg/kg	7.1	10.9	10.5	9.3	10.5	9.9
Fe, mg/kg	228	314	632	213	466	321
Zn, mg/kg	214	2,097	1,903	33	1,940	1,906

[a]NC (negative control, no added Zn source); ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine).

[b]Corn was replaced by ZnO or ZnM (wt/wt) to provide supplemental dietary Zn concentrations of 2,000 mg of Zn/kg of diet.

[c]Provided the following per kilogram of diet: 0.14 mg of I as ethylenediamine dihydroiodide; 3.60 mg of Fe as $FeSO_4$; 2.00 mg of Mn as $MnSO_4$; and 0.30 mg of Se as Na_2SeO_3.

[d]Provided the following per kilogram of diet: 5,511 IU of vitamin A; 551 IU of vitamin D_3; 66 IU of vitamin E; 13.2 mg of vitamin K activity; 4.40 mg of menadione; 36 μg of vitamin B_{12}; 4.40 mg of riboflavin; 17.6 mg of d-panthothenic acid; 26.5 mg of niacin; 1.10 mg of thiamine; and 0.99 mg of pyridoxine.

Housing and Fecal, Urine, and Orts Collection. Pigs were housed in metabolism pens located in a temperature-controlled room. Initial room temperature was maintained at 29°C and decreased by 1°C weekly. Each metabolism pen was constructed of stainless steel, provided 0.81 m^2 of total space, and contained a stainless steel feeder and nipple waterer that allowed for access to feed and water throughout the experiment. Pigs were fed to appetite twice daily. Additionally, each pen was designed to allow for the total but separate collection of urine, feces, and orts. Fecal material was collected daily during the 14-d experiment by removing a screen (1 mm) located directly beneath the entire floor space of the pen. Fecal samples were collected, placed in plastic bags, and stored in a -20°C freezer prior to analysis. Below the fecal collection screen was a stainless steel pan that was angled toward a 10-mm hole on one end. A 4-quart capacity polypropylene container was located at the end of the pan to collect all urine at the same time fecal samples were collected. Total urine volume was recorded daily and a 50-mL subsample was retained in a polypropylene tube and stored at -20°C until analysis. Orts were collected daily at the same time as urine and fecal samples, oven-dried (Fisher Isotemp, Fischer Scientific, Hampton, NH), and used in the determination of ADFI.

Performance, Blood, Tissue, and Whole-body Collection. Pigs were weighed and bled on d 0, 7, and 14. Pig weights were used to calculate ADG. Blood samples were drawn by jugular venipuncture into 10-mL heparinized (143 USP units of sodium heparin per tube) Vacutainer (Becton Dickinson, Franklin Lakes, NJ) tubes with 21 gauge, 3.81-cm needles and then centrifuged at 4°C, 2,000 x g, for 10 min (Beckman GS-6KR, Palo Alto, CA). Plasma was collected into polypropylene tubes and stored at -80°C until mineral analysis.

At the conclusion of the experiment, pigs (mean BW = 8.7 kg; 32 ± 2 d) were fasted and then killed via cardiac injection of sodium pentobarbital (87 mg/kg of BW). Liver and kidney samples were excised and stored in Whirl-Pak bags (Nasco, Fort Atkinson, WI) at -80°C until mineral analysis. The remaining whole-body of each pig was processed as previously described by Rincker et al. (2004). Additionally, six pigs from the original contemporary group (mean BW = 6.1 kg; 18 ± 2 d) were killed prior to the initiation of the balance study. Whole-body samples were collected in a similar manner as that previously described to establish baseline (BL) measurements of mineral concentrations.

Experiment 2

Animals and Treatments. Four sets of six littermate barrows (n = 24; Duroc x Landrace – Yorkshire), with an initial mean BW of 7.2 kg, were weaned at 20 ± 1 d for this 35-d feeding experiment. Four barrows from each litter were randomly allotted, within litter, on the basis of initial BW to one of five dietary treatments. Dietary treatments were obtained by supplementing the basal diets (Table 2) with 0, 25, 50, 100, or 150 mg of Fe/kg of diet as ferrous sulfate monohydrate ($FeSO_4 \cdot H_2O$). The remaining barrow from each litter was allotted to the basal diet supplemented with 0 mg of Fe per kg of diet. There were a total of eight pigs fed the basal diet, and four pigs per treatment fed the basal diet supplemented with 25, 50, 100, or 150 mg of Fe/kg of diet.

Table 2. Composition of basal diets used in Exp. 2 (as-fed basis)

	Diets		
Ingredient, %	Phase 1 (d 0 to 7)	Phase 2 (d 7 to 21)	Phase 3 (d 21 to 35)
Corn[a]	28.23	45.19	59.26
Soybean meal (48% CP)	10.21	20.29	30.70
Whey, spray dried	20.00	10.00	-
Lactose	10.00	5.00	-
Protein plasma	4.00	2.00	-
Egg, spray dried	8.00	4.00	-
Skim milk powder	6.00	2.00	-
Potato protein	5.00	2.00	-
L-Lysine HCl	-	0.15	0.15
Sodium phosphate, monobasic	0.94	1.26	1.49
Calcium sulfate, dihydrate	2.23	2.72	3.08
Sodium chloride	-	-	0.20
Zinc oxide	0.28	0.28	0.01
Copper sulfate	0.01	0.01	0.01
Mineral premix[b]	0.50	0.50	0.50
Vitamin premix[c]	0.60	0.60	0.60
Soybean oil	4.00	4.00	4.00
Analyzed composition			
CP, %	25.98	24.92	26.30
Lysine, %	1.64	1.35	1.32
Ca, %	1.14	1.28	1.08
P, %	0.73	0.75	0.71
Mg, %	0.23	0.29	0.17
Cu, mg/kg	42.90	51.90	14.10
Fe, mg/kg	189.00	223.80	97.80
Zn, mg/kg	1,755.30	1,946.50	107.50
Mn, mg/kg	41.54	58.25	27.92

[a]Corn was replaced by ferrous sulfate monohydrate (wt/wt) to provide supplemental dietary Fe concentrations of 0, 25, 50, 100, and 150 mg of Fe/kg of diet.

[b]Provided the following per kilogram of diet: 0.14 mg of I as ethylenediamine dihydroiodide; 3.60 mg of Fe as $FeSO_4$; 2.00 mg of Mn as $MnSO_4$; and 0.30 mg of Se as Na_2SeO_3.

[c]Provided the following per kilogram of diet: 5,511 IU of vitamin A; 551 IU of vitamin D_3; 66 IU of vitamin E; 13.2 mg of vitamin K activity; 4.40 mg of menadione; 36 μg of vitamin B_{12}; 4.40 mg of riboflavin; 17.6 mg of d-panthothenic acid; 26.5 mg of niacin; 1.10 mg of thiamine; and 0.99 mg of pyridoxine.

Complexity of the diet changed by phase to meet or exceed NRC (1998) nutrient recommendations, excluding Fe. Phase 1 (d 0 to 7) and Phase 2 (d 7 to 21) diets were fed in pelleted form; whereas, Phase 3 (d 21 to 35) diets were fed in meal form. To formulate a diet similar to those used in the commercial industry, yet minimize Fe contributions by ingredients, we analyzed potential feedstuffs for minerals of interest (Table 3). To limit the Fe concentration in the basal diets, the base mineral mix used in this experiment contained minimal Fe (729 mg/kg, as-fed basis) in comparison with typical commercial base mineral mixes which contain 15,000 to 20,000 mg Fe/kg.

Table 3. Analysis of potential dietary ingredients to be used in Exp. 2 (as-fed basis)

	mg/kg					
Ingredient	Cu	Fe	Mn	Zn	Ca, %	P, %
Corn	2136.6	22.1	1.2	13.5	0.03	0.11
Whey, spray dried	0.0	0.0	0.0	5.2	0.37	0.84
Lactose	0.0	5.8	0.0	0.2	1.10	0.70
Skim milk powder	0.1	0.0	0.0	43.1	1.25	1.05
Brewer's yeast	2.7	37.8	8.8	76.6	0.03	1.15
Soybean meal (48% CP)	16.6	148.0	29.6	51.4	0.32	0.41
Fish meal	8.0	705.8	38.9	108.5	5.70	3.16
Poultry meal	35.7	230.4	5.2	99.4	2.61	1.93
Egg, spray dried	1.8	60.9	0.0	43.7	0.36	0.64
Potato protein	38.5	127.6	0.1	14.3	0.05	0.22
Plasma protein	11.5	84.9	0.0	13.9	0.12	1.71
Blood meal, spray dried	7.6	1,493.8	0.0	49.1	0.04	0.10
Blood cells, spray dried	2.1	2,731.2	0.0	15.5	0.02	0.19
Limestone	8.1	425.0	49.6	2.4	44.70	0.13
Monocalcium phosphate	5.5	8,941.1	288.7	99.1	17.79	22.27
Dicalcium phosphate	7.2	7,741.2	264.1	105.1	22.66	19.37
Mineral premix[a]	153.6	728.7	1,038.7	497.6	20.70	0.06

[a]Mineral premix used in Exp. 2

Housing and Fecal, Urine, and Orts Collection. Pigs were housed in the same metabolism pens used in Exp. 1. Pigs were fed their assigned diets and given a 4-d adjustment period followed by a 3-d collection period where urine, feces, and orts were collected (Period 1: d 5 to 7; Period 2: d 12 to 14; Period 3 d 26 to 28). During each dietary collection period, feces and urine samples were collected daily, processed, and stored as described in Exp. 1.

Prior to analysis, daily fecal samples collected in each dietary period (3 d) were combined, mixed, and a composite sample was obtained for each period. A composite urine sample for each pig was obtained by thawing and mixing the daily 50-mL subsamples for each period. A percentage of each daily subsample corresponding to that sample's percentage of the total urine output for that period was used to obtain the composite sample for each period.

Performance, Blood, and Tissue Collection. Pigs were weighed at the end of each dietary phase to calculate ADG. Additionally, pigs were bled on d 0, 7, 21, and 35 via jugular venipuncture as described in Exp. 1. An aliquot of whole blood was transferred into a polypropylene tube and stored on ice for hemoglobin (Hb) and hematocrit (Hct) determination. The remaining whole blood was processed as described in Exp. 1 to collect plasma for mineral analysis.

Laboratory Analysis

In Exp. 2, daily fecal and urine samples were analyzed individually; whereas, composite fecal and urine samples for each dietary period were analyzed in Exp. 2. Fecal samples were oven-dried and then ground in a Cyclotec Mill (1093 Sample Mill, FOSS, Eden Prairie, MN) equipped with a 1-mm screen. Urine samples were prepared for

mineral analysis by centrifugation (500 x g, 4°C, and 10 min) and the supernatant was transferred to a clean polypropylene tube. Feed, fecal, urine, liver, kidney, and whole-body samples were analyzed for minerals (Ca, Cu, Fe, Mg, Mn, P, and Zn) as previously described (Rincker et al., 2004). Analysis of plasma (PCu, PFe, and PZn), whole-body (protein), and blood (Hb and Hct) samples was also performed as previously described (Rincker et al., 2004). All analyses were performed in duplicate. Feed, fecal, and whole-body mineral concentrations were reported on a DM basis; whereas, liver and kidney mineral concentrations were reported on a wet basis.

Statistical Analysis

Performance (ADG), tissue, and whole-body data were analyzed as a randomized complete block design using the MIXED procedures of SAS (SAS Inst. Inc., Cary, NC). The model included the effects of litter (replication), treatment, and litter x treatment (error) with litter considered a random effect. Pig was the experimental unit for analysis of data. Additionally, blood measurements and nutrient balance data were analyzed using the MIXED model methodology of SAS for analysis of repeated measure data. The subject for the repeated measures was individual pig nested within treatment and d 0 Hb, Hct, PCu, PFe, and PZn were used as covariates for their respective individual analyses. Statistical analysis of Zn balance data was performed on $\log_e$-transformed least squares means. Thus, data presented are back-transformed means with error bars for corresponding 95 % confidence intervals. In Exp. 1, two preplanned orthogonal comparisons were made: 1) NC vs. Zn (2,000 mg of Zn/kg of diet as ZnO or ZnM); 2) ZnO vs. ZnM. In Exp. 2, the effects of increasing dietary concentrations of supplemental Fe were partitioned into linear and curvilinear components using orthogonal polynomial

contrasts. Due to unequally spaced dietary concentrations of supplemental Fe, coefficients were derived using the integrative matrix language (PROC IML) procedures of SAS. Differences were considered significant at the level of $P < 0.050$ and highly significant at the level of $P < 0.010$.

Results and Discussion

Experiment 1

In Phase 1 (Table 1), the analyzed Zn concentration in the NC diet was greater than the NRC (1998) recommendation for a 5 to 10 kg pig (100 mg of Zn/kg of diet). Available Zn is greater in animal protein sources than plant protein sources (Baker, 2001). The inclusion of plasma protein in the Phase 1 NC diet resulted in the high Zn concentrations. Replacement of plasma protein with soy protein concentrate in the Phase 2 NC diet resulted in a decrease in Zn concentration. However, the bioavailability of Zn in feedstuffs is influenced by other factors such as phytate or dietary Ca concentration (Underwood and Suttle, 1999).

Growth performance response to pharmacological dietary Zn is variable. Feeding pharmacological Zn concentrations (1,500 to 3,000 mg of Zn/kg of diet) to nursery pigs has been shown to improve growth performance (Carlson et al., 1999; Hill et al., 2000; Hill et al., 2001). However, no benefits were reported due to pharmacological Zn concentrations (3,000 mg of Zn/kg of diet as ZnO) in pigs weaned at 21-d of age (Tokach et al., 1992). In the present experiment, no differences ($P > 0.100$) were observed in growth performance (data not shown). Nursery pigs in an isolated and clean environment and fed diets containing carbadox did not respond to pharmacological Zn (Meyer et al., 2002). Our pigs were individually housed in stainless steel metabolism pens; whereas,

Meyer et al. (2002) fed two pigs per pen. Social facilitation occurs when animals are group housed and may confound experimental results when conducting nutrient balance studies (Brumm and Gonyou, 2001). Pigs in Exp. 1 originated from a herd with a high health status and scouring was not observed during the 14-d experiment. Reports suggest that pharmacological Zn improves growth performance via an improvement in gut morphology (Carlson et al., 1999) and a reduction in scouring (Poulsen, 1992).

Pigs fed the NC diet had greater dietary DM intake during Phases 1 ($P = 0.041$) and 2 ($P = 0.044$) and overall ($P = 0.043$) than pigs fed ZnO or ZnM diets (Table 4). The increase in dietary DM intake for NC fed pigs may have prevented any observed growth differences. However, dietary Zn intake of pigs fed the NC diet during Phase 1 (38 mg of Zn/d) and 2 (10 mg of Zn/d) was below the NRC (1998) recommendation for a 5 to 10 kg pig (50 mg of Zn/d). No clinical signs of Zn deficiency were observed. Other researchers (Hill et al., 1986; Wedekind et al., 1994) have reported adequate growth performance in nursery pigs when dietary Zn concentrations (24 to 33 mg of Zn/kg of diet) were less than the NRC (1998) recommendation.

Table 4. Effects of dietary Zn supplementation on nursery pig daily dietary intake and excretion in Exp. 1[a]

Item	Treatment[b]			SEM	*P*-value[c]	
	NC	ZnO	ZnM		NC vs. Zn	ZnO vs. ZnM
Phase 1, d 0 to 7						
DM intake, g/d	183.30	155.58	115.66	19.20	0.041	0.147
Fecal DM, g/d	9.60	7.34	4.26	2.92	0.283	0.461
Urine, L/d	1.55	1.84	1.46	0.51	0.863	0.603
Phase 2, d 7 to 14						
DM intake, g/d	309.87	282.00	243.51	19.20	0.044	0.162
Fecal DM, g/d	27.07	31.49	22.91	2.92	0.971	0.041
Urine, L/d	2.13	2.30	2.03	0.51	0.957	0.708
Overall, d 0 to 14						
DM Intake, g/d	246.58	218.79	179.59	18.20	0.043	0.140
Fecal DM, g/d	18.34	19.42	13.58	2.82	0.584	0.155
Urine, L/d	1.84	2.07	1.75	0.50	0.909	0.653

[a]Data are least squares means (n = 8 per treatment; initial mean BW = 6.5 kg; initial age = 18 ± 2 d).

[b]NC (negative control, no added Zn source); ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine).

[c]Preplanned orthogonal comparisons were: 1) NC vs. Zn (2,000 mg of Zn/kg of diet as ZnO or ZnM); 2) ZnO vs. ZnM.

Pigs fed pharmacological Zn concentrations as either ZnO or ZnM had greater ($P <$ 0.001) dietary Zn intake than pigs fed the NC diet throughout the 14-d experiment (Figure 1A). Consequently, these pigs had greater ($P < 0.001$) fecal Zn excretion than pigs fed the NC diet during Phase 2 (Figure 1B). The percentage Zn retained from the diet by pigs fed pharmacological ZnO or ZnM diets tended to be greater ($P = 0.063$) during Phase 1 and was greater ($P = 0.002$) during Phase 2 than pigs fed the NC diet (data not shown). After weaning, voluntary feed intake is generally insufficient to meet maintenance requirements (Bark et al., 1986). Pluske et al. (1995) estimated that the energy requirement for maintenance was not met until d 5 after weaning at 21 d of age. Although dietary DM intake during Phase 1 was comparable to values we previously reported in group housed pigs (Rincker et al., 2004), the high percentage Zn retained during Phase 1 (91.87 and 95.47% for ZnO and ZnM, respectively) may indicate an improvement in efficiency to meet the rapid growth while compensating for a limited feed intake.

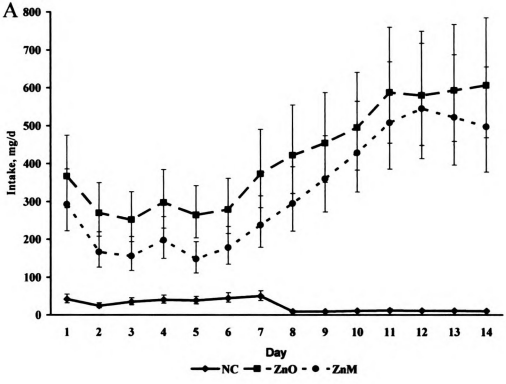
Because dietary Zn intake of pigs fed the NC diet was below the NRC (1998) recommendation, pigs had negligible fecal Zn excretion throughout the 14-d experiment (Figure 1B). Retention of dietary Zn increases when the dietary concentration is below the body's requirement. Thus, the fecal Zn excretion of pigs fed the NC diet is assumed to be of endogenous origin. Endogenous fecal Zn excretion represents an inevitable and constant metabolic loss, as well as, homeostatic mechanisms excreting Zn that was absorbed in excess of the body's requirements (Weigand and Kirchgessner, 1980). They reported that fecal Zn excretion in rats fed diets containing 5.6 or 10.6 mg of Zn/kg of diet was completely of endogenous origin. The Zn present in most plant protein sources

is poorly available because it forms an insoluble complex with phytate (Oberleas et al., 1962). In Phase 2, the low percentage Zn retained (3.76%) in pigs fed the NC diet may be attributed to the low dietary Zn concentration, a large portion of plant protein sources in the diet, and inevitable endogenous losses.

Feces are the major route of Zn excretion in pigs. Feeding 2,000 mg of Zn/kg of diet as ZnO or ZnM resulted in a 12.9- and 9.2-fold increase, respectively, in the overall amount of fecal Zn excreted compared with pigs fed the NC diet (202.5 vs. 2,615.9 and 1,863.1 mg). The greatest increase in fecal Zn excretion occurred during Phase 2 when fecal Zn excretion increased 13- and 57-fold in pigs fed ZnO or ZnM diets, respectively, from d 7 to 14 (Figure 1B). After a 10-d adaptation period to pharmacological Zn diets, Case and Carlson (2002) reported a similar increase (15-fold) in fecal Zn excretion between pigs fed 3,000 vs. 250 mg of Zn/kg of diet as ZnO. Because they did not measure Zn balance during the loading phase (d 0 to 10), Zn balance was negative (-109.0 mg/d) for d 10 to 15. We determined daily Zn balance from weaning until 14 d postweaning. Pigs fed pharmacological ZnO or ZnM diets were in a positive Zn balance throughout Phase 1 (d 0 to 7) and did not approach a negative Zn balance until d 13 to 14 when they became Zn loaded and homeostatic mechanisms increased fecal Zn excretion. Meyer et al. (2002) reported that fecal Zn excretion increased (20-fold) and percentage Zn absorbed decreased as dietary Zn concentration increased (0 to 3,000 mg of Zn/kg of diet as ZnO); however, pigs were not in a negative Zn balance after 21 d.

During Phase 2, pigs fed diets containing 2,000 mg of Zn/kg of diet as ZnM had greater ($P < 0.001$) urinary Zn excretion than pigs fed ZnO or NC diets (Figure 1C). In lambs, Spears (1989) reported that urinary Zn excretion tended to be less with ZnM than

ZnO. They suggested that the disparity in urinary Zn excretion between sources may represent differences in postabsorptive metabolism of Zn. It has been shown in piglets that 48 h after a methionine load, the dose is fully catabolized and excreted as urinary S products (Hou et al., 2003). While all of the diets in Exp. 1 were formulated to contain equal concentrations of methionine, the organic Zn source has Zn bound to methionine, which may result in a greater portion of absorbed Zn being metabolized via the renal system.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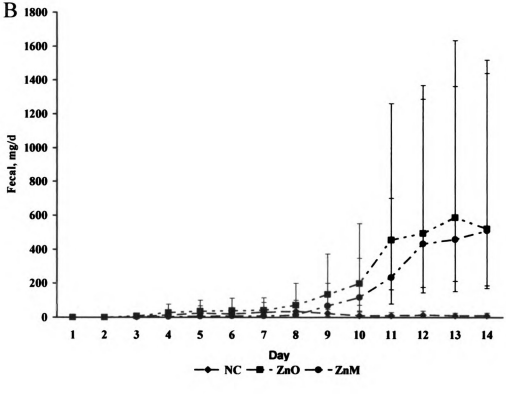
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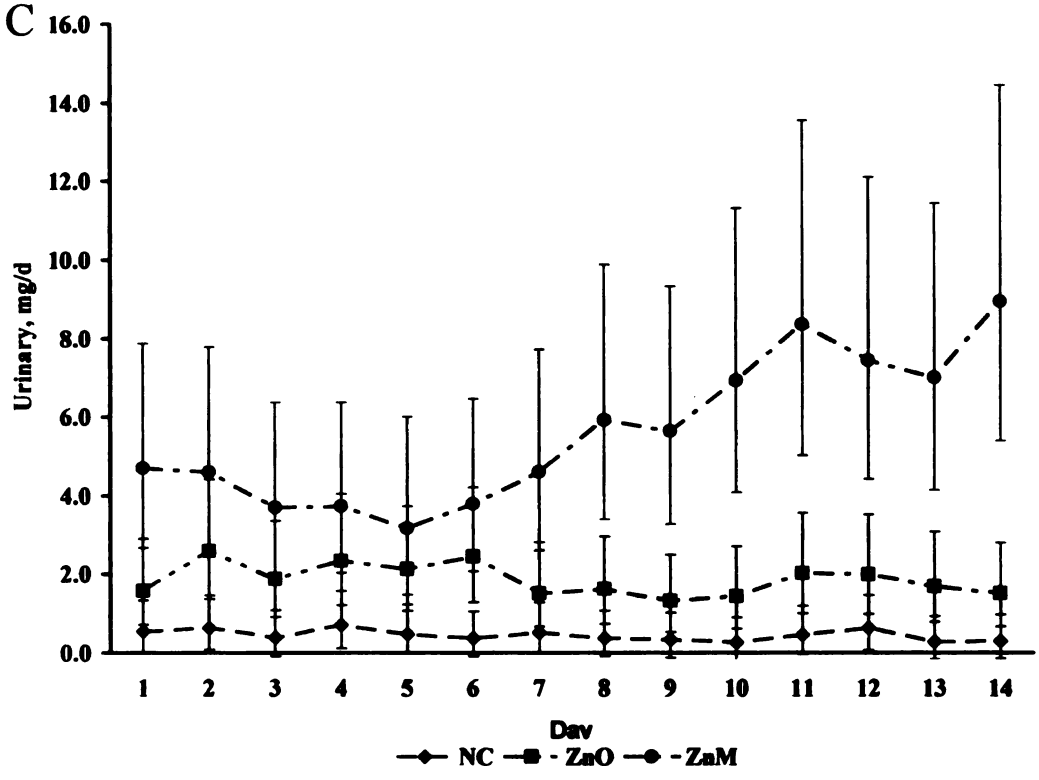

Figure 1. Effects of dietary Zn supplementation in Exp. 1 on nursery pig: A) dietary Zn intake, mg/d (treatment, $P < 0.001$); B) fecal Zn excretion, mg/d (treatment x day, $P = 0.003$); C) urinary Zn excretion, mg/d (treatment, $P < 0.001$). Statistical analysis of data (n = 8 per treatment; initial mean BW = 6.5 kg; initial age = 18 ± 2 d) were performed on $\log_e$-transformed least squares means. Data presented are back-transformed means with error bars for corresponding 95 % confidence intervals. Treatments were: NC (negative control, no added Zn source); ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine).

Pigs fed pharmacological ZnO or ZnM diets had greater ($P = 0.003$) dietary Fe intake and fecal Fe excretion than pigs fed the NC diet (Table 5). Pharmacological ZnO and ZnM diets had greater dietary Fe concentrations (Table 1). Meyer et al. (2002) also reported an increase in fecal Fe excretion in pigs fed pharmacological ZnO (2,000 to 3,000 mg of Zn/kg of diet) for 21 d; however, they noted an increase in dietary Fe concentrations because of the Fe in the commercial Zn oxide source. Pigs fed pharmacological ZnO diets had greater ($P = 0.043$) dietary Cu intake compared with pigs fed pharmacological ZnM diets (Table 5). Because dietary Cu concentrations were similar, this is due to the slightly greater dietary DM intake of pigs fed pharmacological ZnO vs. ZnM diets.

Table 5. Effects of dietary Zn supplementation on nursery pig daily Cu and Fe intake and excretion in Exp. 1[a]

Item	Treatment[b]			SEM	*P*-value[c]	
	NC	ZnO	ZnM		NC vs. Zn	ZnO vs. ZnM
Cu, d 0 to 14						
Intake, mg/d	2.090	2.329	1.808	0.166	0.918	0.043
Fecal, mg/d	1.266	1.527	1.418	0.174	0.311	0.639
Urinary, mg/d	0.009	0.014	0.016	0.003	0.079	0.574
Fe, d 0 to 14						
Intake, mg/d	53.88	90.15	74.57	6.50	0.003	0.112
Fecal, mg/d	24.62	36.92	38.51	5.06	0.053	0.828
Urinary, mg/d	1.01	1.16	1.63	0.26	0.246	0.212

[a]Data are least squares means (n = 8 per treatment; initial mean BW = 6.5 kg; initial age = 18 ± 2 d).

[b]NC (negative control, no added Zn source); ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine).

[c]Preplanned orthogonal comparisons were: 1) NC vs. Zn (2,000 mg of Zn/kg of diet as ZnO or ZnM); 2) ZnO vs. ZnM.

In pigs, the normal range for PZn concentration is 0.8 to 1.2 mg/L (Underwood and Suttle, 1999). In Exp. 1, the initial (d 0) mean PZn concentration was 1.05 mg/L (Table 6). Pigs fed pharmacological ZnO or ZnM diets had a greater PZn concentration on d 7 ($P = 0.018$) and 14 ($P = 0.001$) than pigs fed the NC diet. However, no differences ($P > 0.100$) in PZn concentration were observed in pigs fed the two Zn sources at pharmacological concentrations. Other research has shown that pigs fed 2,000 mg of Zn/kg of diet as ZnM had greater PZn concentration on d 14 than pigs fed 2,000 mg of Zn/kg of diet as ZnO (Schell and Kornegay, 1996). Hahn and Baker (1993) reported that ZnO had a lower uptake from the gut resulting in a lower PZn concentration in contrast to other Zn sources with Zn bound to sulfate, lysine, or methionine. Using a broken-line plot of PZn concentration as a function of supplemental ZnO intake, Hahn and Baker (1993) suggested that PZn concentration was unresponsive when supplemental ZnO intake was less than 1,300 mg/d. Our data suggest otherwise; dietary Zn intake of pigs fed the NC, ZnO, or ZnM diet were 38.2, 296.6, and 190.9 mg/d during Phase 1 and 10.1, 528.4, and 440.6 mg/d during Phase 2, respectively. This is in agreement with Wedekind et al. (1994) who evaluated lower dietary Zn concentrations (27 mg of Zn/kg of basal diet to 47 mg of added Zn/kg of basal diet) and observed a linear response in PZn concentration.

Hill and Matrone (1970) reported that the trace minerals Cu, Fe, and Zn are transition metals that have similar chemical and physical properties (i.e. similar electronic structure). Thus, an imbalance in one mineral can have an antagonistic effect on the concentration of another mineral. In rats, Murthy et al. (1974) reported that a negative correlation existed between PCu and PZn concentration. This is thought to occur via

metallothionein which has a greater affinity for Cu than Zn (Hall et al., 1979). In our study (Table 6), no differences ($P > 0.100$) in PCu and PFe concentrations were observed between experimental treatments even though PZn concentration was increased in pigs fed pharmacological Zn compared with those fed the NC diet. This may be due to the length of the study (14 d).

Table 6. Effects of dietary Zn supplementation on nursery pig plasma mineral concentrations in Exp. 1[a]

	Supplemental Zn[b]				*P*-value[c]	
Item	NC	ZnO	ZnM	SEM	NC vs. Zn	ZnO vs. ZnM
Plasma Cu, mg/L						
d 7	1.58	1.63	1.85	0.11	0.179	0.122
d 14	1.38	1.34	1.38	0.08	0.787	0.537
Plasma Fe, mg/L						
d 7	1.30	1.62	1.66	0.22	0.217	0.871
d 14	0.89	1.04	1.09	0.15	0.290	0.778
Plasma Zn, mg/L						
d 7	0.70	1.40	1.00	0.16	0.018	0.096
d 14	0.47	1.11	1.24	0.09	0.001	0.321

[a]Data are least squares means (n = 8 per treatment; initial mean BW = 6.5 kg; initial age = 18 ± 2 d). Blood concentrations on d 0 were: plasma Cu = 2.07 mg/L (SEM = 0.06); plasma Fe = 0.27 mg/L (SEM = 0.04); plasma Zn = 1.05 mg/L (SEM = 0.04).

[b]NC (negative control, no added Zn source); ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine).

[c]Preplanned orthogonal comparisons were: 1) NC vs. Zn (2,000 mg of Zn/kg of diet as ZnO or ZnM); 2) ZnO vs. ZnM.

Pigs fed pharmacological ZnO or ZnM diets had greater liver Fe ($P = 0.006$) and Zn ($P = 0.001$) concentrations than pigs fed the NC diet (Table 7). Although no differences ($P = 0.633$) in liver Zn concentration were observed between Zn sources in our experiment, Schell and Kornegay (1996) reported that pigs fed 2,000 mg of Zn/kg of diet as ZnM had greater liver Zn concentration than pigs fed 2,000 mg of Zn/kg of diet as ZnO. Excess Zn is known to induce a Cu deficiency, including anemia and decreased ceruloplasmin activity (Magee and Matrone, 1960). Ceruloplasmin, a key Cu-containing enzyme, is required for binding of Fe to transferrin via its ferroxidase activity (Osaki and Johnson, 1969). Consequently, an accumulation of Fe may occur due to a decrease in ceruloplasmin activity (Lee et al., 1968). However, no differences ($P > 0.100$) in liver Cu concentration were observed in this study. Pigs fed the NC diet did have a lesser ($P = 0.002$) kidney Cu concentration and greater kidney Fe ($P = 0.002$) and Zn ($P = 0.001$) concentrations than pigs fed pharmacological ZnO or ZnM diets (Table 7). The greater kidney Zn concentration and lesser urinary Zn excretion in pigs fed the NC diet suggest that these pigs tenaciously retain Zn in renal tissue by minimizing urinary Zn loss to compensate for low available Zn in the diet. Thus, renal Cu and Fe were altered because of the three-way interaction among Cu, Fe, and Zn as previously reported by Hill et al. (1983b). These results are also in agreement with Schell and Kornegay (1996) who reported a greater Fe and lesser Cu concentration in the kidney of pigs fed the control diet (105 mg of Zn/kg of diet) compared with pigs fed 3,000 mg of Zn/kg of diet.

Table 7. Effects of dietary Zn supplementation on nursery pig liver and kidney mineral concentrations (wet basis) in Exp. 1[a]

Item	Treatment[b]			SEM	*P*-value[c]	
	NC	ZnO	ZnM		NC vs. Zn	ZnO vs. ZnM
Liver						
DM, %	26.25	26.05	26.82			
Cu, mg/kg	39.36	40.67	36.82	5.45	0.908	0.545
Fe, mg/kg	130.22	161.90	194.59	12.98	0.006	0.050
Zn, mg/kg	52.52	341.81	373.23	46.50	0.001	0.633
Kidney						
DM, %	19.33	19.00	19.40			
Cu, mg/kg	6.39	16.49	13.75	1.98	0.002	0.321
Fe, mg/kg	39.13	29.83	34.85	2.57	0.002	0.026
Zn, mg/kg	20.67	8.62	8.78	1.07	0.001	0.909

[a]Data are least squares means (n = 8 per treatment; mean BW = 8.7 kg; 32 ± 2 d).

[b]NC (negative control, no added Zn source); ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine).

[c]Preplanned orthogonal comparisons were: 1) NC vs. Zn (2,000 mg of Zn/kg of diet as ZnO or ZnM); 2) ZnO vs. ZnM.

Information regarding the mineral composition of today's swine genetics, which is necessary for nutrient management plans, is limited. Whole-body mineral concentrations of nursery pigs in Exp. 1 are shown in Table 8. Pigs fed pharmacological ZnO or ZnM diets had a greater ($P = 0.001$) whole-body Zn concentration than pigs fed the NC diet. No difference ($P = 0.713$) was observed between Zn sources. We previously reported that pigs fed pharmacological ZnO during Phases 1 (d 0 to 7) and 2 (d 7 to 21) and then adequate Zn (107.5 mg of Zn/kg of diet) during Phase 3 (d 21 to 35) had a mean whole-body Zn concentration of 72.3 mg/kg (Rincker et al., 2004). In Exp. 1, whole-body Zn concentration of pigs fed pharmacological Zn for 14 d was increased 5-fold compared with pigs of similar genetics that were fed pharmacological Zn for 21 d and then adequate Zn for 14 d before being killed (Rincker et al., 2004). This suggests that when pharmacological Zn is removed from the diet, homeostatic mechanisms reduce the excess tissue Zn accumulated during earlier phases.

No differences ($P > 0.100$) were observed in the percentage protein and whole-body Cu, Fe, Mg, Mn, Ca, and P concentrations between pigs fed pharmacological ZnO or ZnM diets and pigs fed the NC diet (Table 8). However, pigs fed the ZnO diet had greater ($P < 0.010$) whole-body Mg and P concentrations compared with pigs fed the ZnM diet. The reduced whole-body Mg and P concentrations in pigs fed pharmacological ZnM may be attributed to a slightly lower dietary DM intake. Mahan and Shields (1998) evaluated the macro- and micro-mineral concentrations of pigs at various intervals from birth to 145 kg BW. They reported that macromineral concentrations generally reflected the metabolic need for soft and (or) hard tissue development, while trace elements, except for Fe and Zn, maintained a fairly constant

concentration from weaning to 145 kg of BW. Increases in the concentration of Fe and Zn paralleled increases in pig weights, reflecting tissue expansion and a corresponding increase in heme compounds and epidermal tissue, respectively. Wiseman et al. (2003) reported that pigs with greater lean gain potential had greater body concentrations of minerals (Zn, Cu, S, and K), particularly those associated with lean tissue deposition.

Table 8. Effects of dietary Zn supplementation on nursery pig whole-body mineral concentration (DM basis) and percentage protein in Exp. 1[a]

Item	Treatment[b]			SEM	*P*-value[c]	
	NC	ZnO	ZnM		NC vs. Zn	ZnO vs. ZnM
Cu, mg/kg	9.15	10.01	9.94	0.48	0.161	0.910
Fe, mg/kg	211.33	199.50	216.77	8.03	0.743	0.169
Mg, mg/kg	978.83	1,013.82	924.16	22.05	0.720	0.012
Mn, mg/kg	3.93	3.14	4.20	0.69	0.690	0.173
Zn, mg/kg	62.23	346.36	358.17	22.23	0.001	0.713
Ca, g/kg	22.82	25.31	22.59	1.32	0.450	0.128
P, g/kg	14.32	15.40	13.52	0.56	0.799	0.011
Protein, %	18.67	18.73	18.62	0.34	0.999	0.781

[a]Data are least squares means (n = 8 per treatment; mean BW = 8.7 kg; 32 ± 2 d). Baseline (n = 6; 6.1 kg; 18 ± 2 d) mineral concentrations and percentage protein were: Cu = 10.92 mg/kg (SEM = 0.52); Fe = 178.13 mg/kg (SEM = 16.65); Mg = 788.36 mg/kg (SEM = 9.53); Mn = 0.90 mg/kg (SEM = 0.12); Zn = 72.38 mg/kg (SEM = 2.10); Ca = 19.42 g/kg (SEM = 1.23); P = 14.57 g/kg (SEM = 0.90); protein = 18.59 % (SEM = 0.37).

[b]NC (negative control, no added Zn source); ZnO (NC + 2,000 mg of Zn/kg of diet as Zn oxide); ZnM (NC + 2,000 mg of Zn/kg of diet as Zn methionine).

[c]Preplanned orthogonal comparisons were: 1) NC vs. Zn (2,000 mg of Zn/kg of diet as ZnO or ZnM); 2) ZnO vs. ZnM.

Experiment 2

In Exp. 2, increasing concentrations of supplemental Fe did not affect ADG (data not shown). Amine et al. (1972a) suggested that BW gain is not a sensitive indicator of Fe adequacy because a decrease in growth is one of the last signs of an Fe disorder following the development of hypochromic-microcytic anemia. Studies by Dove and Haydon (1991), Yu et al. (2000), and Rincker et al. (2004) also reported no effect on growth performance due to reduced dietary Fe concentration.

Hemoglobin and Hct are commonly used to assess Fe status because of their ease of measurement. These indicators of Fe status decrease after the depletion of liver, kidney, and spleen Fe stores. There were no differences ($P > 0.100$) in blood measurements at the start of Exp. 2 (Table 9). Following completion of Phase 2 (d 21), increasing dietary Fe concentration resulted in a linear increase ($P = 0.001$) in Hb concentration and Hct percentage that was maintained until the study ended (d 35). A whole blood Hb concentration of 100 g/L is considered adequate, while 80 g/L suggests borderline anemia, and 70 g/L or less indicates anemia (Zimmerman, 1980). During the 35-d experiment, mean Hb concentration of all experimental treatment groups remained above the adequate concentration. A linear increase in PFe concentration was observed in response to experimental diets on d 7 ($P = 0.034$), 21 ($P = 0.054$), and 35 ($P = 0.011$). Results for blood measurements in Exp. 2 are consistent with previous results from our lab using a larger number of pigs (Rincker et al., 2004).

As previously mentioned, an interrelationship exists between Cu, Fe, and Zn because of their similar chemical and physical properties (Hill and Matrone, 1970). On d 35, a linear increase ($P = 0.045$) in PZn concentration was observed in response to the increase

in dietary Fe concentration (Table 9). Flanagan et al. (1980) reported that dietary Fe deficiency enhanced the absorption of Zn in rats. However, pigs in Exp. 2 were not Fe deficient as evident by their Hb concentration. Even though PZn concentration was affected by dietary treatments, plasma mineral concentrations were within normal ranges (PZn: 0.8 to 1.2 mg/L; PCu: 1.0 to 1.3 mg/L) reported by Underwood and Suttle (1999).

Table 9. Effects of dietary Fe supplementation on nursery pig hematological status and plasma mineral concentrations in Exp. 2[a]

	Supplemental Fe, mg/kg of diet						*P*-value[b]	
Item	0	25	50	100	150	SEM	Linear	Quad
Hemoglobin, g/L								
d 7	116.45	112.77	113.58	113.52	121.53	4.33	0.309	0.176
d 21	111.58	115.62	116.87	126.80	135.18	4.33	0.001	0.723
d 35	111.82	126.53	127.95	134.37	136.07	4.33	0.001	0.062
Hematocrit, %								
d 7	46.98	45.07	46.43	45.18	47.62	1.59	0.688	0.301
d 21	45.29	46.89	48.53	50.13	53.25	1.59	0.001	0.963
d 35	45.08	51.24	52.28	54.05	54.37	1.59	0.001	0.022
Plasma Cu, mg/L								
d 7	1.46	1.44	1.43	1.55	1.51	0.11	0.475	0.970
d 21	1.21	1.29	1.41	1.28	1.50	0.11	0.108	0.963
d 35	1.26	1.38	1.38	1.07	1.51	0.11	0.503	0.218
Plasma Fe, mg/L								
d 7	0.99	1.52	2.79	1.34	2.35	0.33	0.034	0.223
d 21	1.22	1.48	2.39	2.06	2.06	0.33	0.054	0.073
d 35	0.68	1.76	2.09	1.67	2.16	0.33	0.011	0.105
Plasma Zn, mg/L								
d 7	1.20	1.42	1.24	1.23	1.31	0.08	0.912	0.979
d 21	1.26	1.50	1.44	1.33	1.46	0.08	0.425	0.634
d 35	0.90	1.00	0.90	0.99	1.12	0.08	0.045	0.498

[a]Data are least squares means (n = 8 pigs fed the basal diet supplemented with 0 mg of Fe per kg of diet and for other treatments n = 4; initial mean BW = 8.2 kg; initial age = 20 ± 1 d). Blood levels on d 0 were: hemoglobin = 114.49 g/L (SEM = 4.26); hematocrit = 45.76% (SEM = 1.47); plasma Cu = 2.14 mg/L (SEM = 0.12); plasma Fe = 0.43 mg/L (SEM = 0.06); plasma Zn = 1.20 mg/L (SEM = 0.13).

[b]Linear and quadratic effects of increasing dietary Fe concentration.

The improvements in dietary DM intake during Periods 2 (linear, $P = 0.002$) and 3 (quadratic, $P = 0.025$) were of sufficient magnitude that dietary DM intake tended to increase (linear, $P = 0.075$) during the 35-d experiment (Table 10). A similar response to treatments was observed in fecal DM excretion during Periods 2 (linear, $P = 0.003$) and 3 (quadratic, $P = 0.055$); however, no differences ($P > 0.100$) were observed in overall fecal DM excretion.

Table 10. Effects of dietary Fe supplementation on nursery pig dietary intake and excretion in Exp. 2[a]

	Supplemental Fe, mg/kg of diet						*P*-value[b]	
Item	0	25	50	100	150	SEM	Linear	Quad
Period 1, d 5 to 7								
DM Intake, g/d	251.8	320.7	259.3	277.4	312.8	42.1	0.449	0.819
Fecal DM, g/d	14.4	23.0	14.3	20.4	23.8	5.5	0.220	0.815
Urine, L/d	3.8	2.8	2.0	2.1	1.6	0.7	0.024	0.300
Period 2, d 12 to 14								
DM Intake, g/d	431.8	410.6	486.8	523.7	559.7	42.1	0.002	0.877
Fecal DM, g/d	47.8	50.1	45.5	58.5	63.7	5.5	0.003	0.419
Urine, L/d	3.5	3.4	3.1	2.7	2.0	0.7	0.041	0.799
Period 3, d 26 to 28								
DM Intake, g/d	429.5	467.3	487.2	521.2	411.2	42.1	0.872	0.025
Fecal DM, g/d	41.2	29.6	40.0	46.8	25.7	5.5	0.245	0.055
Urine, L/d	5.2	4.6	2.5	5.2	2.1	0.7	0.013	0.763
Overall[c]								
DM Intake, g/d	371.0	399.5	411.1	440.8	427.9	35.9	0.075	0.276
Fecal DM, g/d	34.5	34.2	33.2	41.9	37.7	4.0	0.122	0.631
Urine, L/d	4.2	3.6	2.5	3.3	1.9	0.6	0.003	0.816

[a]Data are least squares means (n = 8 pigs fed the basal diet supplemented with 0 mg of Fe per kg of diet and for other treatments n = 4; initial mean BW = 8.2 kg; initial age = 20 ± 1 d).

[b]Linear and quadratic effects of increasing dietary Fe concentration.

[c]Overall represents the least squares means of the three balance periods.

Increasing the dietary concentration of supplemental Fe resulted in a linear increase in ($P = 0.001$) dietary Fe intake and fecal Fe excretion (Table 11). However, when expressed as a percentage of intake, there were no differences ($P > 0.100$) in the percentage Fe retained between experimental treatments (data not shown).

Increasing dietary Fe concentration resulted in a linear increase in dietary Zn ($P = 0.003$), Mn ($P = 0.010$), P ($P = 0.070$), and Mg ($P = 0.065$) intake (Table 11). Because diets were formulated to contain equal mineral concentrations, the increases in dietary mineral intake are due to an increase in dietary DM intake. Pigs had increased fecal Zn (linear, $P = 0.020$) excretion and decreased urinary Zn (quadratic, $P = 0.010$), Mn (linear, $P = 0.007$), Ca (linear, $P = 0.031$), and Mg (linear, $P = 0.015$) excretion in response to increasing dietary Fe. The decreases in urinary mineral excretion are the result of a decrease in overall urine volume for the 35-d experiment. The increases in dietary mineral intake combined with the decreases in urinary mineral excretion resulted in a linear increase in Mn ($P = 0.021$), Ca ($P = 0.034$), P ($P = 0.024$), and Mg ($P = 0.014$) retained (data not shown). However, no differences ($P > 0.100$) in the percentage Cu, Fe, Mn, Zn, Ca, and P retained were observed between dietary treatments in Exp. 2 (data not shown).

Table 11. Effects of dietary Fe supplementation on nursery pig mineral intake and excretion in Exp. 2[a]

	Supplemental Fe, mg/kg of diet						*P*-value[b]	
Item[c]	0	25	50	100	150	SEM	Linear	Quad
Fe								
Intake, mg/d	62.1	76.4	87.8	117.6	132.2	9.7	0.001	0.422
Fecal, mg/d	28.4	30.4	35.9	56.0	60.2	5.7	0.001	0.742
Urinary, mg/d	0.7	0.9	1.1	0.8	0.8	0.1	0.785	0.097
Zn								
Intake, mg/d	443.9	479.2	492.0	541.0	595.8	53.0	0.003	0.945
Fecal, mg/d	259.5	291.7	259.9	339.8	352.8	46.1	0.020	0.892
Urinary, mg/d	0.3	0.6	0.5	0.5	0.2	0.2	0.439	0.010
Cu								
Intake, mg/d	13.08	11.96	12.30	11.61	13.73	1.07	0.441	0.032
Fecal, mg/d	6.81	6.29	5.25	6.42	6.94	0.92	0.597	0.126
Urinary, mg/d	0.01	0.02	0.02	0.01	0.01	0.01	0.710	0.118
Mn								
Intake, mg/d	15.90	16.60	15.82	17.13	19.85	1.59	0.010	0.200
Fecal, mg/d	8.36	7.46	7.29	9.29	9.59	1.23	0.103	0.432
Urinary, mg/d	0.22	0.31	0.26	0.17	0.12	0.04	0.007	0.232
Ca								
Intake, g/d	4.35	4.95	4.83	5.31	4.99	0.43	0.119	0.154
Fecal, g/d	1.08	0.85	0.98	1.37	1.06	0.15	0.271	0.558
Urinary, g/d	0.46	0.42	0.33	0.39	0.27	0.07	0.031	0.929
P								
Intake, g/d	2.71	2.91	2.98	3.22	3.12	0.26	0.070	0.291
Fecal, g/d	0.77	0.68	0.73	0.94	0.75	0.09	0.359	0.397
Urinary, g/d	0.14	0.19	0.17	0.13	0.12	0.03	0.216	0.379
Mg								
Intake, g/d	0.88	0.92	0.94	1.01	1.01	0.09	0.065	0.615
Fecal, g/d	0.40	0.40	0.39	0.51	0.46	0.06	0.148	0.765
Urinary, g/d	0.15	0.15	0.11	0.12	0.08	0.02	0.015	0.925

[a]Data are least squares means of the three balance periods (n = 8 pigs fed the basal diet supplemented with 0 mg Fe per kg of diet and for other treatments n = 4; initial mean BW = 8.2 kg; initial age = 20 ± 1 d).

[b]Linear and quadratic effects of increasing Fe concentration.

In conclusion, feeding pharmacological concentrations of Zn to nursery pigs increases Zn stores, but fecal Zn excretion is increased only after 9 to 10 d of supplementation. Results indicate that nursery pigs load Zn in tissues for approximately 9 to 10 d, and then excrete large amounts of Zn resulting in a negative Zn balance after d 13 to 14. In Exp. 2, indicators of hematological status were increased due to supplemental Fe. Additionally, increasing the dietary Fe concentration increased dietary mineral intake via an increase in dietary DM intake. Subsequently, fecal Fe and Zn excretion were increased. The current experiments provide useful data to swine producers regarding whole-body mineral composition, as well as, fecal and urinary mineral excretion values for current genetics.

Implications

Manure management in intensive production units will continue to challenge the swine industry. Consequently, there is a need to continue to evaluate nutritional strategies that maximize animal performance while minimizing environmental concerns. Feeding pharmacological zinc to nursery pigs for nine to ten days has the potential to maintain rapid growth performance without large amounts of fecal zinc being excreted. When pharmacological zinc is fed for a longer duration, tissues become loaded and homeostatic mechanisms excrete excess zinc. Homeostatic mechanisms also regulate the tissue loading of other minerals so that when diets contain more than is required by the body, excess mineral quantities are excreted.

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