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# Iron methionine as a source of iron for the neonatal pig<sup>1</sup>

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## Abstract

Neonatal pigs (143) were used to determine if adequate iron (Fe) would be stored following a single oral dose of Fe methionine (FeMet) to prevent anemia through 21 d of age. Treatments consisted of: control (no Fe), 200 mg injectable Fe as gleptoferron within 12 h of birth (at birth), 100 or 200 mg Fe as FeMet orally at birth, or 200 mg Fe as FeMet or FeSO<sub>4</sub> orally on d 3. Pigs given injectable Fe had higher hemoglobin concentrations than other treatments at 14 and 21 d. Utilization of Fe from FeMet was greatest when given at 3 d compared to at birth. Based on hemoglobin concentrations at 21 d, relative bioavailability of Fe from FeMet given on d 3 was 180% compared to FeSO<sub>4</sub>. Iron methionine given orally was a safe, effective source of Fe, but a single oral dose of FeMet was not equivalent to injectable Fe. © 2002 Elsevier Science Inc. All rights reserved.

*Keywords:* Iron; Anemia; Pig

## 1. Introduction

Confinement reared pigs develop an iron (Fe) deficiency anemia (hypochromic, microcytic) during the first weeks of life. There are several reasons for this including: 1) pigs are born with unusually small Fe stores, 2) milk contains low levels of Fe, and 3) pigs have a very rapid growth rate [1]. Anemic pigs grow slowly, are listless, and are more susceptible

<sup>1</sup> Use of trade names in this publication does not imply endorsement by the North Carolina ARS or criticism of similar products not mentioned.

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to infectious diseases [2]. Therefore, neonatal pigs are usually injected with a chelated form of Fe within 3 d of age to prevent anemia. Oral supplementation would be less invasive and potentially less stressful than injectable Fe for neonatal pigs.

Before the development of injectable Fe compounds, Fe salts, including ferrous sulfate, and ferrous fumarate were used as oral supplements; however, repeated doses were required to maintain adequate hemoglobin concentrations [3]. Braude et al. [4] estimated that a pig must retain 21 mg Fe/kg live weight increase to maintain Fe status. The current National Research Council's [2] requirement for postnatal pigs is slightly lower at 7 to 16 mg Fe/day. Oral Fe is absorbed according to metabolic need and availability is dependent on the Fe compound's solubility in the gastrointestinal tract. [1]

The newborn pig can also absorb large organic molecules intact from the small intestine by pinocytosis for the first few hours of life prior to gut closure [5]. Giving pigs Fe dextran orally within the first 12 h of life was as effective as intramuscular administration of Fe dextran in preventing anemia [6]. Thoren-Tolling [3] also found that oral Fe dextran was more available to neonatal pigs than oral ferrous fumarate.

Iron methionine is another Fe complex that may be an effective source of oral Fe for the young piglet. The objectives of this study were: 1) to determine if adequate amounts of Fe would be stored following a single oral dose of Fe methionine to prevent piglet anemia through 21 d of age and 2) to determine the safety of Fe methionine when administered orally to young pigs.

## **2. Methods and materials**

Twelve litters (n = 143 pigs) from crossbred sows, housed in farrowing crates with plastic coated slatted floors in an environmentally controlled building, were used in this study. Litter size was equalized shortly after birth. Eleven litters contained 12 pigs each and one litter contained 11 pigs. Within 12 h after birth, pigs were stratified by weight within a litter and randomly assigned by weight to treatments.

Treatments consisted of: 1) negative control (no supplemental Fe; n = 24), 2) positive control (intramuscular injection of 200 mg Fe as gleptoferron within 12 h of birth; n = 23), 3) 100 mg Fe as Fe methionine orally within 12 h of birth (n = 24), 4) 200 mg Fe as Fe methionine orally within 12 h of birth (n = 24), 5) 200 mg Fe as Fe methionine orally at 3 d of age (n = 24) and 6) 200 mg Fe as ferrous sulfate orally at 3 d of age (n = 24). Gleptoferron (Gleptosil, Continental Animal Health, Division of Burns-Biotec, Omaha, NE) is a macromolecular complex of beta-ferric oxyhydroxide and dextran glucoheptonic acid and was the positive control in this study. The efficiency of Fe utilization from gleptoferron is similar to Fe dextran in the young pig [7]. Iron methionine (FeMet; Zinpro Corp., Eden Prairie, MN) and ferrous sulfate (FeSO<sub>4</sub>; Fisher, Pittsburgh, PA) were dissolved in saline and administered orally in a volume of 3 mL using a plastic tube.

Pigs were not creep fed, and were observed daily for morbidity and mortality. Pigs were weighed shortly after birth and at 21 d of age (weaning). Blood samples were obtained via vena cava puncture at 0 (prior to treatment), 7, 14 and 21 d of age for hemoglobin and hematocrit determination. Plasma Fe was measured on blood samples obtained on d 7.

Table 1  
Effect of Fe source and route and timing of administration on growth and mortality in pigs

Treatment	Initial weight, kg	Final weight, kg	21-day gain, kg	Mortality, % <sup>3</sup>
Control	1.34	5.07 <sup>a</sup>	3.74 <sup>a</sup>	8
Injectable Fe	1.39	5.91 <sup>b</sup>	4.45 <sup>b</sup>	17
100 mg FeMet <sup>1</sup> at birth <sup>2</sup>	1.40	5.14 <sup>a</sup>	3.77 <sup>a</sup>	14
200 mg FeMet at birth <sup>2</sup>	1.37	5.15 <sup>a</sup>	3.73 <sup>a</sup>	9
200 mg FeMet on d 3	1.35	5.08 <sup>a</sup>	3.72 <sup>a</sup>	0
200 mg FeSO <sub>4</sub> on d 3	1.32	5.52 <sup>a,b</sup>	4.16 <sup>a,b</sup>	32
Standard error	0.06	0.21	0.19	

<sup>1</sup> Fe methionine.

<sup>2</sup> Given within 12 h of birth.

<sup>3</sup> Treatment ( $P < 0.05$ ) by Fishers Exact Test.

<sup>a</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ ) as assessed by F-protected LSD.

<sup>b</sup> See footnote a.

Twenty-four pigs (one randomly selected pig from each treatment from four randomly selected litters) were killed by electrocution on d 21. The liver and spleen were removed from these animals for Fe analysis. All experimental procedures, care, and handling of animals were approved by the North Carolina State University Institutional Animal Care and Use Committee.

Iron in plasma and tissues was determined by atomic absorption spectrophotometry (Model 5000 Perkin Elmer, Norwalk, CT). Tissue samples were prepared for mineral analysis by blotting free of visible blood, drying in a forced air oven at 100°C, then wet ashing with nitric acid in a microwave (MDS8ID, CEM Corp., Matthews, NC). Hemoglobin was determined using a commercially available kit (Sigma Chemical Co., St. Louis, MO). Hematocrit was determined using microcapillary tubes (Autocrit Ultra 3, Beckman Dickerson, Parsippany, NJ).

Weight gain, blood, and tissue data were statistically analyzed by analysis of variance as a completely randomized design using the General Linear Models Procedures of SAS [8]. Differences between treatment means were determined using the LSD test protected by a significant F-value. Differences in mortality were examined with a Fisher Exact test.

### 3. Results

Initial weights (Table 1) were similar across treatments. Body weights at 21 d of age and gains during the 21-d study were greater ( $P < 0.05$ ) for pigs given injectable Fe compared to negative controls or pigs that received FeMet orally. Gains for pigs that received FeSO<sub>4</sub> orally were intermediate and did not differ significantly from pigs given injectable Fe, FeMet orally, or negative controls.

Percentage mortality during the 21-d study is also shown in Table 1. Two pigs that received 100 mg and one pig that received 200 mg of Fe from FeMet within 12 h of birth

Table 2  
Effect of Fe source, and route and timing of administration on hematocrits (%) in pigs

Treatment	Day 1	Day 7	Day 14	Day 21
Control	29.5	22.6 <sup>a</sup>	20.1 <sup>a</sup>	19.6 <sup>a</sup>
Injectable Fe	31.6	33.8 <sup>b</sup>	37.2 <sup>d</sup>	37.7 <sup>d</sup>
100 mg FeMet <sup>1</sup> at birth <sup>2</sup>	31.3	31.7 <sup>b</sup>	29.2 <sup>b</sup>	26.9 <sup>b,c</sup>
200 mg FeMet at birth <sup>2</sup>	32.1	32.8 <sup>b</sup>	29.2 <sup>b</sup>	26.9 <sup>b,c</sup>
200 mg FeMet on d 3	30.9	31.5 <sup>b</sup>	32.7 <sup>c</sup>	28.8 <sup>c</sup>
200 mg FeSO <sub>4</sub> on d 3	30.6	32.5 <sup>b</sup>	27.8 <sup>b</sup>	24.5 <sup>b</sup>
Standard error	0.96	0.87	0.91	1.06

<sup>1</sup> Fe methionine.

<sup>2</sup> Given within 12 h of birth.

<sup>a</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ ) as assessed by F-protected LSD.

<sup>b</sup> See footnote a.

<sup>c</sup> See footnote a.

<sup>d</sup> See footnote a.

died shortly after processing. These pigs were not included in the mortality data because it was assumed that their death was related to stress involved in handling, weighing, ear notching and Fe administration rather than FeMet per se. Two pigs in the FeSO<sub>4</sub> treatment and one pig in the FeMet treatment died shortly after Fe was administered on d 3. Death of these pigs was attributed to the Fe being administered into the lungs. These pigs also were not included in the mortality results. Percentage mortality (treatment effect [ $P < 0.05$ ]) was higher in pigs given FeSO<sub>4</sub> orally compared to negative and positive controls and pigs given oral FeMet.

Hematocrits did not differ on d 1 (Table 2). By d 7 hematocrits were lower ( $P < 0.05$ ) in negative control pigs compared to those receiving supplemental Fe. Hematocrits were similar on d 7 in pigs given Fe regardless of Fe level or source and time of administration. Pigs that received injectable or oral Fe continued to have higher ( $P < 0.05$ ) hematocrits throughout the 21-d study than negative control pigs. However, pigs that received injectable Fe had higher ( $P < 0.05$ ) hematocrits on d 14 and 21 than those given the oral Fe treatments. Pigs given 200 mg of Fe as FeMet on d 3 had higher ( $P < 0.05$ ) hematocrits on d 14 and 21 than pigs given a similar quantity of Fe as FeSO<sub>4</sub> on d 3. Hematocrits were greater ( $P < 0.05$ ) on d 14 and tended to be higher ( $P = 0.19$ ) on d 21 in pigs given FeMet on d 3 compared to those given FeMet within 12 h of birth. No differences in hematocrits were observed between pigs given 100 and 200 mg of Fe from FeMet shortly after birth.

Hemoglobin concentrations did not differ on d 1 (Table 3). Negative control pigs had much lower ( $P < 0.05$ ) hemoglobin concentrations than pigs given supplemental Fe on d 7, 14 and 21. On d 7, pigs that received FeMet on d 3 had lower ( $P < 0.05$ ) hemoglobin concentrations than pigs given injectable Fe. On d 7, hemoglobin concentrations of pigs given other Fe treatments did not differ from pigs injected with Fe. Hemoglobin concentrations were lower in pigs ( $P < 0.05$ ) given all oral Fe treatments compared to pigs that received injectable Fe on d 14 and 21. On d 14 and 21, pigs that received FeSO<sub>4</sub> had lower ( $P < 0.05$ ) hemoglobin concentrations than pigs given FeMet on d 3. Pigs that received

Table 3  
Effect of Fe source, and route and timing of administration on hemoglobin concentrations (g/dL) in pigs

Treatment	Day 1	Day 7	Day 14	Day 21
Control	9.65	7.05 <sup>a</sup>	5.65 <sup>a</sup>	5.31 <sup>a</sup>
Injectable Fe	9.87	10.80 <sup>c</sup>	11.59 <sup>d</sup>	11.47 <sup>d</sup>
100 mg FeMet <sup>1</sup> at birth <sup>2</sup>	9.79	10.21 <sup>b,c</sup>	9.00 <sup>b</sup>	7.71 <sup>b,c</sup>
200 mg FeMet at birth <sup>2</sup>	9.65	10.60 <sup>b,c</sup>	8.98 <sup>b</sup>	7.71 <sup>b,c</sup>
200 mg FeMet on d 3	9.75	9.74 <sup>b</sup>	10.11 <sup>c</sup>	8.56 <sup>c</sup>
200 mg FeSO <sub>4</sub> on d 3	9.66	10.62 <sup>b,c</sup>	8.30 <sup>b</sup>	7.12 <sup>b</sup>
Standard error	0.32	0.35	0.30	0.39

<sup>1</sup> Fe methionine.

<sup>2</sup> Given within 12 h of birth.

<sup>a</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ ) as assessed by F-protected LSD.

<sup>b</sup> See footnote a.

<sup>c</sup> See footnote a.

<sup>d</sup> See footnote a.

FeMet shortly after birth had lower ( $P < 0.05$ ) hemoglobin concentrations on d 14 and tended to have lower ( $P = 0.12$ ) concentrations on d 21 compared to pigs given FeMet on d 3.

Plasma Fe concentrations were measured on blood samples collected on d 7 (Table 4). Negative control pigs had very low plasma Fe concentrations by d 7 ( $P < 0.05$ ). Pigs given injectable Fe and oral FeMet on d 3 had higher ( $P < 0.05$ ) plasma Fe concentrations than animals given oral FeMet within 12 h of birth or oral FeSO<sub>4</sub> on d 3.

Iron concentrations in liver and spleen on d 21 were much greater ( $P < 0.05$ ) in pigs that received injectable Fe (Table 5). On d 21, liver and spleen Fe concentrations in pigs given oral Fe did not differ from negative control pigs.

Table 4  
Effect of Fe source and route and timing of administration on plasma Fe concentrations ( $\mu\text{g/ml}$ ) in pigs on day 7

Treatment	Plasma Fe
Control	0.17 <sup>a</sup>
Injectable Fe	2.04 <sup>c</sup>
100 mg FeMet <sup>1</sup> at birth <sup>2</sup>	0.98 <sup>b</sup>
200 mg FeMet at birth <sup>2</sup>	0.90 <sup>b</sup>
200 mg FeMet on d 3	1.72 <sup>c</sup>
200 mg FeSO <sub>4</sub> on d 3	0.74 <sup>b</sup>
Standard error	0.15

<sup>1</sup> Fe methionine.

<sup>2</sup> Given within 12 h of birth.

<sup>a</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ ) as assessed by F-protected LSD.

<sup>b</sup> See footnote a.

<sup>c</sup> See footnote a.

Table 5

Effect of Fe source and route and timing of administration on liver and spleen Fe concentrations (mg/kg dry tissue) in pigs

Treatment	Liver Fe	Spleen Fe
Control	95 <sup>a</sup>	370 <sup>a</sup>
Injectable Fe	642 <sup>b</sup>	968 <sup>b</sup>
100 mg FeMet <sup>1</sup> at birth <sup>2</sup>	133 <sup>a</sup>	336 <sup>a</sup>
200 mg FeMet at birth <sup>2</sup>	114 <sup>a</sup>	337 <sup>a</sup>
200 mg FeMet on d 3	99 <sup>a</sup>	395 <sup>a</sup>
200 mg FeSO <sub>4</sub> on d 3	87 <sup>a</sup>	301 <sup>a</sup>
Standard error	46	70

<sup>1</sup> Fe methionine.

<sup>2</sup> Given within 12 hours of birth.

<sup>a</sup> Means in the same column with different superscript letters are significantly different ( $P < 0.05$ ) as assessed by F-protected LSD.

<sup>b</sup> See footnote a.

Estimated bioavailability of 200 mg Fe from FeMet relative to FeSO<sub>4</sub> is shown in Table 6. Values were calculated using the increase in hematocrits, hemoglobin concentrations, and plasma Fe concentrations above that were observed in control pigs not supplemented with Fe. Relative bioavailability of Fe from FeMet given shortly after birth was 133 to 149% that of FeSO<sub>4</sub>. When FeMet was given on d 3, relative bioavailability of Fe was 180 to 277% compared to FeSO<sub>4</sub>.

#### 4. Discussion

The results of this study indicate that FeMet can be safely administered orally to pigs either within a few hours of birth or at 3 d of age. Adverse effects of oral Fe administration that have been reported, such as diarrhea were not observed in the present study.

The tendency for slightly higher gains in FeSO<sub>4</sub> treated pigs compared to those that received FeMet can be explained by the high mortality rate in the FeSO<sub>4</sub> treatment. A large percentage of the poor performing pigs in the FeSO<sub>4</sub> treatment died during the study and are therefore, not included in the treatment mean for gain. Pigs that received injectable Fe were

Table 6

Relative bioavailability of Fe methionine compared to ferrous sulfate

	Hematocrit <sup>3</sup>	Hemoglobin <sup>3</sup>	Plasma Fe <sup>4</sup>
200 mg FeMet <sup>1</sup> at birth <sup>2</sup>	149%	133%	136%
200 mg FeMet on d 3	188%	180%	277%

<sup>1</sup> Fe methionine.

<sup>2</sup> Given within 12 hours of birth.

<sup>3</sup> Data collected on d 21.

<sup>4</sup> Data collected on d 7.

heavier at 21-d than pigs that received no Fe or 100 or 200 mg Fe as oral FeMet. This agrees with other studies [7,9] that demonstrated that injectable Fe improved gains to 21 d, relative to control pigs given no supplemental Fe. In contrast, Maxwell and coworkers [10] observed no effect on average daily gain through 21 d due to injection of 100 mg Fe dextran versus oral supplementation of 250 mg Fe as FeMet within 24 h of birth.

Iron injected as gleptoferron was well utilized based on hematocrits and hemoglobin concentrations throughout the study. Pigs injected with 200 mg Fe as gleptoferron also had considerable storage Fe remaining in their liver at the end of the 21-d study.

The utilization of Fe from FeMet was greatest when given at 3 d. This finding was somewhat surprising, because it was expected that FeMet would be readily absorbed early in life via pinocytosis. After gut closure, which should be complete before 3 d of age, a much lower percentage absorption of Fe would be expected, especially from a large dose (200 mg) of Fe. The current study is in contrast to Kallella and Karlsson [11] who demonstrated that ferrous fumarate, administered orally, prevented anemia better when given at 3 to 8 h after birth as compared to 3 d after birth. More than 70% of plasma Fe turnover is used for red cell production within 2 weeks [12]. Higher concentrations of plasma Fe on d 7 concurs with greater hematocrits and hemoglobin concentrations on d 14 and 21 in pigs given 200 mg Fe as FeMet on d 3.

Iron status throughout the study was similar in pigs receiving 100 or 200 mg FeMet within 12 h of birth. This indicates that these neonatal pigs had a limited ability to either absorb or store Fe during the first few hours of life. However, Furugouri and Kawabata [13,14] found no differences in the Fe absorptive capability of the small intestine from pigs at birth or 3 d. They proposed that the neonatal pig intestine fully provides active absorptive function of Fe from birth. Utilization of Fe in the body, whether for hemoglobin synthesis or storage in the liver, requires oxidation and reduction of Fe (ferric $\leftrightarrow$ ferrous). Iron is absorbed as ferrous Fe; both forms of oral Fe used are the ferrous state. For Fe to bind to transferrin, the protein that transports Fe in the body, it must be in the ferric state. Iron bound to ferritin (the major Fe storage protein) also is in the ferric form while Fe in hemoglobin exists in the ferrous form [1]. The enzyme ceruloplasmin is important in the oxidation of ferrous to ferric Fe and this enzyme is absent or very low at birth [15]. The molybdeno-enzyme, xanthine dehydrogenase, is proposed to be the primary ferroxidase in mucosa [1]. No data was found on the activity of this enzyme early in life. This and other enzymes involved in the storage or utilization of Fe may be low at birth and develop following parturition.

Hemoglobin concentrations were higher in pigs given FeMet at birth compared to negative controls, clearly indicating that Fe from FeMet was used for hemoglobin synthesis. However, if the Fe from the large dose of FeMet given shortly after birth had been efficiently stored, hemoglobin concentrations would not have dropped as rapidly at 14 and 21 d. A limited ability to store Fe after absorption from FeMet given within 12 h of birth may have resulted in the FeMet complex being excreted in the urine, or sloughed off in enterocytes.

Utilization of FeMet given at 3 d was probably limited somewhat by the pig's ability to absorb FeMet. However, bioavailability of Fe from FeMet given at 3 d was 170 to 277% that of FeSO<sub>4</sub>. In earlier research, approximately 60% of a single oral dose of 30 mg labeled Fe as FeSO<sub>4</sub> was retained up to 240 h after dosing; however, by 384 h this was approximately 40% [4]. Using radiolabeled Fe dextran given 3 to 6 h after birth, 21% of the Fe was present

5 d after dosing, at 19 d the radioiron depot in the liver was almost depleted in pigs that received Fe dextran orally, but about 7% of the dose was still recovered in the liver of the pigs that received an i.m. dose [3]. A similar study injecting 100 mg Fe as Fe dextran compared to 250 mg oral Fe as FeMet on d 1 showed no effect on hemoglobin or hematocrit on d 7, but by d 14 and 21 hematocrit and hemoglobin were lower in the pigs given oral Fe [10].

Hemoglobin concentrations of 10 g/dL are considered normal, 8 g/dL indicates borderline anemia, while 6 g/dL indicates severe anemia [16]. Using this criterion in the present study, the negative control pigs were severely anemic by d 14, and with the exception of pigs given 200 mg of FeMet on d 3, pigs given the oral Fe treatments were marginally anemic on d 21.

In summary, FeMet given orally was a safe and readily available source of Fe for the neonatal pig. However, limited absorption and/or storage of the oral Fe resulted in Fe stores not sufficient to meet the Fe requirements for 21 d. Based on the results of this study a second dose of FeMet at approximately 10 d of age would be required to maintain maximal hemoglobin concentrations until weaning at 21 d. Iron methionine may have been utilized more efficiently if the complex had been given just prior to gut closure (30–36 h). This would have allowed more time for enzymes involved in Fe utilization to increase. Further studies are needed to determine the optimal time(s) for FeMet administration and to develop a convenient, safe delivery system for administering FeMet orally.

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