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Cover Page Footnote

Appreciation is expressed to Dr. James Usry, Micronutrients USA, LLC., Indianapolis, IN for technical and financial support.

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Effects of Feeding Increasing Levels of Iron from Iron Sulfate or Iron Carbonate on Nursery Pig Growth Performance and Blood Criteria¹

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Summary

A total of 140 weanling pigs (DNA 241 × 600, initially 12.2 ± 0.02 lb) were used in a 32-d study evaluating the effects of increasing dietary iron from either iron sulfate (FeSO₄) or a micronized, agglomerated ferrous carbonate (FeCO₃) on nursery pig growth performance and blood criteria. The micronized form of FeCO₃ is designed to improve nursery pig growth performance and blood iron status. Pigs used for this trial did not receive an iron injection after birth in order to increase sensitivity to added dietary iron. Pigs were weaned at approximately 21 d and were allotted to pens based on initial BW in a randomized complete block design with 5 pigs in each pen and 4 pens per treatment. Experimental treatments were arranged as a 2 × 3 + 1 factorial with main effects of dietary iron source (FeSO₄ vs. FeCO₃) and level (10, 30, or 50 ppm) plus a negative control with no additional dietary iron. The basal diet was formulated to contain 40 ppm total dietary iron based on ingredient contributions and was formulated with an iron-free trace mineral premix. Experimental diets were formulated below the pigs' recommended iron requirement based on National Research Council (NRC)⁴ estimates. Experimental diets were fed in pellet form for the duration of the trial. From d 0 to 32, there were no iron source × level interactions observed. Increasing iron improved (linear; $P < 0.05$) average daily gain (ADG), average daily feed intake (ADFI), feed efficiency (F/G), hemoglobin (Hgb), and hematocrit (Hct). There was no evidence of difference ($P > 0.10$) for an iron source effect on growth performance or blood criteria measured. Therefore, either iron source can be used in diets fed to weanling pigs without affecting performance.

¹Appreciation is expressed to Dr. James Usry, Micronutrients USA, LLC., Indianapolis, IN for technical and financial support.

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⁴NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press., Washington, D.C.

Introduction

Iron is an essential mineral that is involved in numerous cellular functions such as DNA synthesis and oxidative phosphorylation that are crucial for maintaining normal body metabolism.⁵ More importantly, iron is a major component of hemoglobin in red blood cells. The inadequate absorption of iron reduces the number of circulating red blood cells resulting in anemia and poor growth performance.⁶ Rincker et al. observed that the amount of iron contributed by feed ingredients alone was not sufficient to maintain iron status above anemic levels. However, increasing iron sulfate (FeSO_4) in nursery pig diets improved growth performance and iron status.⁷ Ferrous carbonate (FeCO_3 ; Micronutrients USA, LLC., Indianapolis, IN) is a micronized, agglomerated form of iron that has the potential for improved bioavailability. We are not aware of any previous research that describes the effects of feeding this micronized, agglomerated form of FeCO_3 to pigs. Therefore, the objective of this study was to determine the effects of increasing levels of FeSO_4 or FeCO_3 on nursery pig growth performance and blood criteria.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS.

A total of 140 nursery pigs (DNA 241 $\times$ 600, initially 12.2 lb BW) were used in a 32-d study with 5 pigs per pen and 4 replications per treatment. Each pen (4 $\times$ 4 ft) had metal tri-bar flooring, one 4-hole self-feeder, and a nipple waterer to provide *ad libitum* access to feed and water. Pigs were weaned at approximately 21 d of age and allotted to pens based on initial BW in a completely randomized design to 1 of 7 dietary treatments. Prior to the start of this trial, pigs were not administered an iron injection at processing during lactation to ensure pigs were sensitive to the dietary treatments.

The treatments were arranged in a 2 $\times$ 3 + 1 factorial with main effects of added dietary iron source (FeSO_4 vs. FeCO_3) and level (10, 30, or 50 ppm) plus a negative control with no additional dietary iron. The iron sources were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1). The iron sulfate source contained 30% iron and the ferrous carbonate source contained 37% iron.

All diets were corn-milk byproduct based and balanced for amino acids according to NRC⁴ requirement estimates. Treatment diets were fed in a single phase. Feed ingredients were analyzed for iron content prior to formulation and values were used to formulate the dietary treatments. The basal diet was formulated to contain 40 ppm total dietary iron based on ingredient contributions and was formulated with an iron-free trace mineral premix. Treatment diets were formulated below the pigs' recommended

⁵Linder, M.C. 1991. Nutrition and metabolism of the trace elements. *Nutr. Biochem. Met.* 2:215-276

⁶Kim, J.C., Wilcox, P., and M. R. Bedford. 2017. Iron status of piglets and impact of phytase superdosing on iron physiology: a review. *Anim. Feed Sci. Tech.* 235:8-14.

⁷Rincker, M. J., G. M. Hill, J. E. Link, and J. E. Rowntree. 2004. Effects of dietary iron supplementation on growth performance, hematological status, and whole-body mineral concentrations of nursery pigs. *Journal of Animal Science* 82:3189-3197.

iron requirement based on the NRC⁴ estimates. A 1:1 Ca to P ratio was utilized to limit inclusion of calcium carbonate because it also contains iron. Diets were fed in pelleted form and were prepared at the Kansas State University O.H. Kruse Feed Technology Innovation Center, Manhattan, KS. Diet samples were collected from every bag at manufacturing and 6 pooled samples of each dietary treatment were submitted for analysis of dry matter (DM), crude protein (CP), Ca, P, and iron in duplicate (Ward Laboratories, Inc., Kearney, NE; Table 2).

Pigs and feeders were weighed on d 0, 7, 14, 21, and 32 to determine ADG, ADFI, and F/G. All pigs were bled on the same day that they were weighed. Blood was collected via jugular venipuncture, and blood was analyzed for hemoglobin (Hgb) and hematocrit (Hct) at the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS.

Growth data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. The main effects of iron source and linear and quadratic effects of level, as well as their interactions were evaluated using preplanned CONTRAST statements. Blood data were analyzed as a repeated measure with pen as the experimental unit. The main effects of iron source, day, treatment, and linear and quadratic effects of level, as well as their interactions, were evaluated using preplanned CONTRAST statements. Differences between treatments were determined by using least squares means. A P -value ≤ 0.05 was considered significant and $0.05 < P \leq 0.10$ was considered marginally significant.

Results and Discussion

Results of the diet analysis indicated that all diets analyzed had higher Ca and P compared to the formulated values, while DM and CP closely matched formulated values (Table 2). Iron analysis of the diets indicated that the control diet and the diet with 50 ppm added iron from FeCO_3 were higher than expected, with other diets similar to calculated values.

From d 0 to 32, no evidence of difference ($P > 0.10$) was observed for a source $\times$ level interaction or source effect for all growth performance criteria (Table 3). Average daily gain, ADFI, F/G, and final BW was improved (linear; $P < 0.05$) with increasing iron from either FeSO_4 or FeCO_3 .

For Hgb, there was no evidence of difference ($P > 0.10$) observed for a treatment $\times$ day or source $\times$ level interaction at any of the collection time points (Table 4). There was also no evidence of difference ($P > 0.10$) observed for an iron source effect at any of the collection time points. A day effect was observed in which all Hgb values increased ($P = 0.001$) throughout the study. There was no evidence of difference ($P > 0.10$) in Hgb values on d 0 and 7 of the study. Hemoglobin values improved (linear; $P < 0.05$) with increasing dietary addition of either FeSO_4 or FeCO_3 on d 14, 21, and 32.

For Hct, no evidence of difference ($P > 0.10$) was observed for a treatment $\times$ day interaction or source effect at any of the time points measured. A day effect was observed in which all Hct values increased ($P = 0.001$) throughout the study. There was no evidence

of difference ($P > 0.10$) in Hct values on d 0 and 7 of the study. Marginal significance ($P = 0.089$) was observed for a source $\times$ level interaction on d 21. This interaction was the result of pigs fed diets with 50 ppm of added FeSO_4 having greater Hct values than pigs being fed diets with 50 ppm of added FeCO_3 on d 21. Hematocrit values improved (linear; $P < 0.05$) with increasing dietary addition of either FeSO_4 or FeCO_3 on d 14, 21, and 32.

Interestingly, we observed that the control pigs that were fed diets without supplemental iron had an increase in Hgb and Hct from d 0 to 32. This is reflective of the pigs' ability to obtain supplemental iron from the basal dietary ingredients in enough quantities to influence body iron status as determined by circulating Hgb and Hct. This is also in agreement with Williams et al. who also showed that in the absence of an iron injection early in life, pigs fed common nursery diets after weaning had improved iron status.⁸

In summary, these data suggest that the two sources of dietary iron influenced nursery pig growth performance and blood criteria similarly. This suggests that the micronized form of FeCO_3 is a sufficient alternative source of iron that can be added to nursery diets. Similar to previous research, increasing added iron improved growth performance and increased Hgb and Hct criteria when pigs have low iron status at weaning.

⁸Williams, H.; Woodworth, J. C.; DeRouchey, J. M.; Dritz, S. S.; Tokach, M. D.; Goodband, R. and A. Holtcamp. 2018. "Effects of increasing iron dosage in newborn pigs on preweaning and subsequent nursery performance," Kansas Agricultural Experiment Station Research Reports: Vol. 2: Iss. 9.

Table 1. Basal diet composition (as-fed basis)¹

Ingredient, %	
Corn	54.52
Soybean meal, 47% crude protein	7.54
Casein	1.30
Skim milk powder	34.00
Calcium carbonate	0.78
Sodium chloride	0.43
Phosphoric acid, 85% ²	0.43
L-lysine HCl	0.31
DL-methionine	0.17
L-threonine	0.16
L-tryptophan	0.03
Vitamin premix	0.25
Trace mineral premix ³	0.10
Iron sulfate monohydrate ⁴	+/-
Ferrous carbonate ^{5,6}	+/-
Total	100
Calculated analysis	
Standardized ileal digestible (SID) AA, %	
Lysine	1.40
Methionine:lysine	41
Methionine and cysteine:lysine	58
Threonine:lysine	63
Tryptophan:lysine	18
Valine:lysine	69
Total lysine, %	1.51
Net energy, kcal/lb	1,171
Crude protein, %	22.3
Calcium, %	0.68
Phosphorus, %	0.68
STTD P, ⁷ %	0.55

¹A total of 140 pigs (DNA 241 × 600) were used in a single-phase nursery trial with 5 pigs per pen and 4 replications per treatment.

²ThermoFisher Scientific, Waltham, MA.

³An iron-free trace mineral premix (University of Auburn, Auburn, AL) was used in place of normal trace mineral premix to decrease iron content of the diet.

⁴Corn was replaced with an equivalent amount of FeSO₄ at 0.07, 0.20, and 0.33% of the diet to form dietary treatments.

⁵Micronutrients, LLC., Indianapolis, IN.

⁶Corn was replaced with an equivalent amount of FeCO₃ at 0.05, 0.16, and 0.27% of the diet to form dietary treatments.

⁷Standardized total tract digestible phosphorus.

Table 2. Chemical analysis of experimental diets¹

Item	Control ³	FeSO ₄ , ppm ⁴			FeCO ₃ , ppm ⁵		
		10	30	50	10	30	50
Dry matter, %	88.2	88.2	87.9	87.8	87.4	87.3	88
Crude protein, %	20.8	20.9	21.7	21.7	21.5	21.7	22.0
Calcium, %	0.91	0.84	0.84	0.84	0.83	0.79	0.85
Phosphorus, %	0.74	0.73	0.70	0.71	0.71	0.69	0.67
Iron, ppm	50.0	55.1	72.5	87.4	46.4	67.1	109.6

¹An iron-free trace mineral premix was used in place of normal trace mineral premix to decrease iron content of diet. Complete diet samples were obtained from each dietary treatment during manufacturing. Samples of diets were pooled into 6 individual composite samples and then submitted for analysis of DM, CP, Ca, P, and iron (Midwest Laboratories, Inc., Omaha, NE) in duplicates.

³Negative control that was formulated to contain 40 ppm total iron in the diet.

⁴Iron sulfate added at 10, 30, or 50 ppm of Fe.

⁵Ferrous carbonate (Micronutrients USA, LLC., Indianapolis, IN) added at 10, 30, or 50 ppm of iron.

Table 3. Effects of increasing iron sulfate or ferrous carbonate on nursery pig growth performance¹

Item ²	Control ³	FeSO ₄ , ppm ⁴			FeCO ₃ , ppm ⁵			Probability, <i>P</i> <				
		10	30	50	10	30	50	SEM	Source × level	Source	Level	
											Linear	Quadratic
d 0 to 32												
ADG, lb	0.25	0.42	0.37	0.53	0.40	0.31	0.52	0.05	0.875	0.452	0.001	0.578
ADFI, lb	0.45	0.56	0.59	0.66	0.57	0.54	0.68	0.04	0.952	0.856	0.001	0.790
F/G	1.90	1.34	1.60	1.27	1.47	1.78	1.37	0.14	0.925	0.244	0.018	0.919
BW, lb												
d 0	12.2	12.2	12.2	12.2	12.2	12.2	12.2	0.02	0.200	0.292	0.605	0.643
d 32	21.5	27.3	26.7	28.7	25.4	23.2	31.1	1.74	0.188	0.450	0.001	0.877

¹A total of 140 pigs (DNA 241 × 600) were used in a 1-phase nursery trial with 5 pigs per pen and 4 replications per treatment.

²ADG = average daily gain. ADFI = average daily feed intake. F/G = feed efficiency. BW = body weight.

³Negative control that was formulated to contain 40 ppm iron.

⁴Iron sulfate added at 10, 30, or 50 ppm of Fe.

⁵Ferrous carbonate (Micronutrients USA, LLC., Indianapolis, IN) added at 10, 30, or 50 ppm of iron.

Table 4. Effects of increasing iron sulfate or ferrous carbonate on nursery pig blood parameters¹

		Probability, $P <$										
Item ²	Control ³	FeSO ₄ , ppm ⁴			FeCO ₃ , ppm ⁵			SEM	Source × level	Source	Level	
		10	30	50	10	30	50				Linear	Quadratic
Hgb, g/dl ^{6,7}												
d 0	4.6	4.5	4.4	4.7	4.4	4.3	4.7	0.21	0.792	0.742	0.695	0.187
d 7	4.6	4.8	4.7	5.2	4.7	4.8	4.7	0.21	0.440	0.426	0.279	0.840
d 14	5.1	5.5	5.3	6.1	5.3	5.5	5.7	0.23	0.635	0.595	0.006	0.522
d 21	5.6	6.0	5.8	7.0	6.2	6.1	6.5	0.28	0.145	0.881	0.006	0.702
d 32	6.9	7.3	7.6	8.5	7.1	7.4	8.2	0.55	0.717	0.193	0.001	0.674
Hct, % ^{6,7}												
d 0	16.6	16.2	15.6	16.9	15.7	15.3	16.4	0.62	0.855	0.596	0.949	0.137
d 7	16.9	17.7	16.9	19.6	17.2	17.6	17.1	0.79	0.248	0.258	0.139	0.578
d 14	18.5	20.3	19.1	22.3	19.6	20.0	20.8	0.85	0.588	0.647	0.005	0.630
d 21	19.8	21.9	20.9	25.6	22.3	21.9	23.4	0.99	0.089	0.737	0.001	0.637
d 32	24.8	26.6	26.9	29.8	26.0	26.5	29.5	1.69	0.993	0.385	0.001	0.923

¹A total of 140 pigs (DNA 241 × 600) were used in a 1-phase nursery trial with 5 pigs per pen and 4 replications per treatment. All pigs on trial were bled and blood was analyzed for hemoglobin and hematocrit (Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Kansas State University, Manhattan, KS).

²Hgb = hemoglobin. Hct = hematocrit.

³Negative control that contained 40 ppm total iron.

⁴Iron sulfate added at 10, 30, or 50 ppm of iron.

⁵Ferrous carbonate (Micronutrients USA, LLC., Indianapolis, IN) added at 10, 30, or 50 ppm of iron.

⁶No evidence of difference (*P* > 0.10) observed for a treatment × day interaction.

⁷Day (*P* < 0.001).