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Non Ruminant Nutrition

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Effects of feeding increasing levels of iron from iron sulfate or iron carbonate on nursery pig growth performance and hematological criteria

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Abstract

A total of 140 weanling pigs (241 × 600, DNA, Columbus, NE; initially 5.5 ± 0.79 kg body weight) were used in a 32-d study evaluating the effects of increasing dietary Fe from either iron sulfate ($FeSO_4$) or iron carbonate ($FeCO_3$) on nursery pig growth performance and blood Fe status. The pigs used for this trial did not receive an Fe injection after birth in order to increase the sensitivity to added dietary Fe after weaning. Pigs were weaned at approximately 21 d and allotted to pens based on the initial weight in a completely randomized block design with five pigs in each pen and four pens per treatment. Experimental treatments were arranged as a 2 × 3 + 1 factorial with main effects of dietary Fe source ($FeSO_4$ vs. $FeCO_3$) and level (10, 30, or 50 mg/kg of added Fe) plus a negative control with no additional dietary Fe. The basal diet contained 40 mg/kg total dietary Fe based on ingredient contributions and was formulated with an Fe-free trace mineral premix. Experimental diets were formulated below the pigs recommended Fe requirement based on NRC (2012) estimates. Experimental diets were fed in pellet form in a single phase for the duration of the trial. From day 0 to 32, there was no evidence for source × level interactions for growth performance, hemoglobin (**Hb**), or hematocrit (**Hct**) values. There was no evidence for a difference ($P > 0.10$) in dietary Fe source. Providing increasing Fe levels in the diet from either $FeSO_4$ or $FeCO_3$ improved ($P < 0.05$) average daily gain, average daily feed intake, gain-to-feed ratio, and increased ($P < 0.05$) Hb and Hct values. A day effect ($P = 0.001$) was observed for both Hb and Hct with values increasing throughout the study. Increasing dietary Fe levels in the diet from either $FeSO_4$ or $FeCO_3$ increased (linear; $P < 0.05$) Hb and Hct values on days 14, 21, and 32. In summary, these data suggest that the micronized form of $FeCO_3$ is a source of Fe that can be added to nursery diets to yield similar responses to those observed from $FeSO_4$ supplementation. Similar to previous research, increasing dietary Fe improved the growth performance and increased Hb and Hct values when pigs have low Fe status at weaning.

Key words: growth performance, hematocrit, hemoglobin, iron carbonate, iron sulfate, nursery pig

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BW	body weight
CP	crude protein
DM	dry matter
DMT-1	divalent metal transporter 1
EDTA	ethylenediaminetetraacetic acid
G:F	gain-to-feed
Hb	hemoglobin
Hct	hematocrit

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 metabolism. More importantly, Fe plays a critical role in the transport and storage of oxygen (Beard, 2001). To prevent anemia in preweaned swine, an exogenous source of injectable Fe is commonly administered. Injectable Fe has consistently been shown to prevent anemia and support growth in suckling pigs (Peters and Mahan, 2008; Chevalier, 2019). Like the suckling pig, the nursery pig is characterized by a rapid growth rate and increased blood volume. The NRC (2012) Fe requirement estimate for nursery pigs from 7 to 11 kg and 11 to 25 kg is 100 mg/kg of the diet to support postweaning growth and blood Fe status. Inorganic Fe sources such as iron sulfate ($FeSO_4$) are commonly added as part of a trace mineral premix to nursery diets, and these inorganic sources are routinely provided at or above the NRC (2012) nursery pig requirement estimates (Flohr et al., 2016). The NRC (2012) further suggests that common feed ingredients supplied in the diet contain enough Fe to meet these requirement estimates. However, the availability of Fe from these feed ingredients may be limited suggesting that the total Fe content of the diet is not reflective of available Fe for the pig (Rincker et al., 2004).

Iron absorption mainly occurs in the lumen of the duodenum. The divalent metal transporter 1 (**DMT-1**) transport protein is the main facilitator of ferrous (Fe^{2+}) Fe entry into enterocytes of the duodenum (Conrad and Umbreit, 2002). The absorbed Fe is bound to the ferritin protein in the enterocytes' cytosol or transported via ferroportin into circulation (Xue and Shah, 2013). The transport via ferroportin is regulated by hepcidin, which is a regulatory hormone that helps maintain Fe homeostasis (Ganz, 2013). Inorganic sources of Fe, such as $FeSO_4$, could be of concern as its absorption and availability may be decreased due to antagonism between trace elements, such as copper and zinc (Umbreit, 2005). Iron carbonate ($FeCO_3$; Micronutrients USA, LLC., Indianapolis, IN) is a relatively novel form of $FeCO_3$; thus, recent research is limited on its effects in nursery pigs. Therefore, the objective of this study was to evaluate the effects of increasing dietary Fe from either $FeSO_4$ or $FeCO_3$ on nursery pig growth performance and blood criteria.

Materials and Methods

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.

Animals

A total of 140 weanling pigs (241 × 600, DNA, Columbus, NE; initially, 5.5 ± 0.79 kg body weight [**BW**]) were used in a 32-d study. The pigs used for this trial did not receive an Fe injection after birth in order to increase the sensitivity to added dietary Fe after weaning. Pigs were weaned at approximately 21 d of age and allotted to pens based on the weight in a completely randomized block design to one of seven dietary treatments with five pigs per pen and four pens per treatment. Dietary treatments were arranged as a 2 × 3 + 1 factorial with main effects of added dietary Fe source ($FeSO_4$ vs. $FeCO_3$) and level (10, 30, or 50 mg/kg) plus a negative control with no added Fe. The Fe sources were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1). The $FeSO_4$ source contained 30% Fe and the $FeCO_3$ source contained 37% Fe as per the manufacturer's specifications. The amount of the Fe source added to the diets were adjusted based on the amount of Fe contained in the two sources. Each pen (1.52 × 1.52 m) had galvanized metal tri-bar flooring, one four-hole self-feeder, and a nipple waterer to provide ad libitum access to feed and water.

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

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%[1]	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[2]	0.10
Iron sulfate monohydrate[3]	+/–
Iron carbonate[4]	+/–
Total	100
Calculated analysis	
Standardized ileal digestible amino acid, %	
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/kg	2,581
CP, %	22.3
Calcium, %	0.68
Phosphorous, %	0.68
Standardized total tract P[5], %	0.55

[1]Thermofisher scientific, Waltham, MA.
[2]An Fe-free trace mineral premix (University of Auburn, Auburn, AL) was used to decrease Fe content of the diet.
[3]Corn replaced with an equivalent amount of $FeSO_4$ (Prince Agri Products, LLC., Teaneck, NJ) at 0.07%, 0.20%, and 0.33% of the diet to form dietary treatments.
[4]Corn replaced with an equivalent amount of $FeCO_3$ (Micronutrients, LLC., Indianapolis, IN) at 0.05%, 0.16%, and 0.27% of the diet to form dietary treatments.
[5]Standardized total tract digestible phosphorous.

The water source used in the experiment originated from a municipal water source and contained approximately 0.03 ppm Fe. Pigs and feeders were weighed on days 0, 7, 14, 21, and 32 to determine average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain-to-feed ratio (**G:F**).

Diet preparation

All diets were prepared at the Kansas State University O.H. Kruse Feed Technology and Innovation Center located in Manhattan, KS. Diets were corn–milk byproduct based in an effort to minimize the total dietary Fe content. Phosphoric acid (Thermofisher Scientific, Waltham, MA) was used as the P source in the diets in place of monocalcium phosphate to minimize the total dietary Fe content. Diets were balanced for amino acids according to NRC (2012) requirement estimates. Experimental diets were fed in pellet form in a single phase for the duration of the trial. Feed ingredients were analyzed for their Fe content prior to formulation and these values used to calculate the Fe content of the basal diet. The basal diet was calculated to contain 40 mg/kg total dietary Fe based on ingredient contributions. All experimental diets were formulated with an Fe-free trace mineral premix and were formulated below the pigs recommended Fe requirement based on the NRC (2012) estimates.

Chemical analysis

Complete diet samples were obtained from each dietary treatment during manufacturing. Six individual samples of each dietary treatment were pooled into a composite, subsampled, and sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for analysis of dry matter (**DM**; AOAC, 2012; method 935.29), crude protein (**CP**; method 990.03; AOAC, 2012), Ca (method 965.14/985.01; AOAC, 2012), and P (method 965.17/985.01; AOAC, 2012). Dietary Fe was analyzed using a Thermo Fisher ICAP-OES 7400 Duo (inductively coupled argon plasma-optical emissions spectrometry) according to Kovar (2003) and AOAC (2012; method 999.11).

Blood analysis

Blood samples were collected via jugular venipuncture in 5-mL ethylenediaminetetraacetic acid (**EDTA**) and whole blood (Monoject, Covidien, Dublin, Republic of Ireland) tubes using 22-gauge, 2.54 cm needles from all 140 pigs on days 0, 7, 14, 21, and 32. Hematological criteria measured included: hemoglobin (**Hb**) and hematocrit (**Hct**) using an ADVIA 2021i Hematology System (Siemens Healthcare Diagnostics, Tarrytown, NY). Blood samples were processed at the Kansas State University Veterinary Diagnostic Lab located in Manhattan, KS.

Statistical analysis

Growth data were analyzed as a completely randomized block design using the GLIMMIX procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and block as a random effect. Treatment served as the fixed effect in the analysis. The main effects of Fe source ($FeSO_4$ vs. $FeCO_3$) and linear and quadratic effects of level (0, 10, 30, and 50 mg/kg added Fe), as well as their interactions, were evaluated using preplanned CONTRAST statements. Results were considered significant at $P \leq 0.05$.

Hematological criteria were analyzed as a repeated measure using the GLIMMIX procedure of SAS 9.4 with pen as the experimental unit and block as a random effect. Treatment served as the fixed effect. The main effects of Fe source, day, treatment, and linear and quadratic effects of level, as well as their interactions, were evaluated using preplanned CONTRAST statements. Results were considered significant at $P \leq 0.05$.

Results

Chemical analysis

The results of the diet analysis indicated that all diets had higher Ca compared with the formulated values, while DM and CP closely matched the formulated values (Table 2). Iron analysis of the diets indicated that the control diet and diet with 50 mg/kg added Fe from $FeCO_3$ were higher than expected, with other diets similar to calculated values.

Growth performance

From day 0 to 32, no evidence of difference ($P > 0.05$) was observed for source × level interactions or source effects for all growth performance criteria (Table 3). ADG, ADFI, G:F, and final weight were increased (linear; $P < 0.05$) with increasing Fe from either $FeSO_4$ or $FeCO_3$.

Hematological criteria

For Hb and Hct, there was no evidence of difference ($P > 0.05$) observed for treatment × day interactions (Table 4).

Table 2. Chemical analysis of experimental diets[1]

		$FeSO_4$, mg/kg[3]			$FeCO_3$, mg/kg[4]		
Item	Control[2]	10	30	50	10	30	50
DM, %	88.2	88.2	87.9	87.8	87.4	87.3	88
CP, %	20.8	20.9	21.7	21.7	21.5	21.7	22.0
Ca, %	0.91	0.84	0.84	0.84	0.83	0.79	0.85
P, %	0.74	0.73	0.70	0.71	0.71	0.69	0.67
Fe, mg/kg	50.0	55.1	72.5	87.4	46.4	67.1	109.6

[1]An Fe-free trace mineral premix was used in place of normal trace mineral premix to decrease the Fe content of diet. Complete diet samples were obtained from each dietary treatment during manufacturing. Six individual samples of each dietary treatment were pooled into a composite, subsampled, and sent to a commercial laboratory (Ward Laboratories, Inc., Kerney, NE) for analysis of DM, CP, Ca, P, and Fe in duplicates.
[2]Calculated to contain 40 mg/kg total Fe in the diet.
[3]Iron sulfate (Prince Agri Products, LLC., Teaneck, NJ) added at 10, 30, or 50 mg/kg of Fe.
[4]Iron carbonate (Micronutrients USA, LLC., Indianapolis, IN) added at 10, 30, or 50 mg/kg of Fe.

Table 3. Effects of increasing iron sulfate or iron carbonate on nursery pig growth performance[1]

									Probability, *P*-value <			
		$FeSO_4$, mg/kg[3]			$FeCO_3$, mg/kg[4]						Level	
Item	Control[2]	10	30	50	10	30	50	SEM	Source × Level	Source	Linear	Quadratic
Day 0 to 32												
ADG, g	113	190	158	240	179	141	236	22.0	0.875	0.452	0.001	0.731
ADFI, g	203	245	267	298	260	243	307	19.7	0.952	0.972	0.001	0.944
G:F	0.549	0.752	0.628	0.798	0.686	0.588	0.754	0.052	0.925	0.185	0.014	0.846
BW, kg												
Day 0	5.5	5.5	5.5	5.5	5.5	5.5	5.5	0.01	0.200	0.474	0.938	0.358
Day 32	9.8	12.4	12.1	13.0	11.5	10.5	14.1	0.79	0.188	0.450	0.001	0.967

[1]A total of 140 pigs (DNA 241 × 600) were used in a one-phase nursery trial with five pigs per pen and four replications per treatment.
[2]Formulated to contain 40 mg/kg of total Fe.
[3]Iron sulfate (Prince Agri Products, LLC., Teaneck, NJ) added at 10, 30, or 50 mg/kg of Fe.
[4]Iron carbonate (Micronutrients USA, LLC., Indianapolis, In) added at 10, 30, or 50 mg/kg of Fe.

Table 4. Effects of increasing iron sulfate or ferrous carbonate on nursery pig blood criteria[1]

									Probability, *P*-value <			
		$FeSO_4$, mg/kg[3]			$FeCO_3$, mg/kg[4]						Level	
Item	Control[2]	10	30	50	10	30	50	SEM	Source × Level	Source	Linear	Quadratic
Hb, g/dl[5,6]												
Day 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
Day 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
Day 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
Day 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
Day 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, %[5,6]												
Day 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
Day 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
Day 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
Day 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
Day 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

[1]A total of 140 pigs (DNA 241 × 600) were used in a one-phase nursery trial with five pigs per pen and four replications per treatment. All pigs on trial were bled, and blood was analyzed for Hb and Hct (Kansas State University Veterinary Diagnostic Lab, Manhattan, KS).
[2]Formulated to contain 40 mg/kg of total Fe.
[3]Iron sulfate added at 10, 30, or 50 mg/kg of Fe.
[4]Ferrous carbonate (Micronutrients USA, LLC., Indianapolis, IN) added at 10, 30, or 50 mg/kg of Fe.
[5]No evidence of difference ($P > 0.10$) observed for a treatment × day interaction.
[6]Day effect ($P < 0.001$).

Also, there was no evidence of difference ($P > 0.05$) observed for a Fe source effect at any of the blood collection time points for Hb and Hct. A day effect was observed in which all Hb and Hct values increased ($P = 0.001$) throughout the study. For dietary Fe level effect, there was no evidence of difference ($P > 0.05$) in Hb or Hct values on days 0 and 7, but Hb and Hct values increased (linear; $P < 0.05$) with increasing dietary addition of either $FeSO_4$ or $FeCO_3$ on days 14, 21, and 32.

Discussion

Iron is one of the most abundant trace minerals found in the body of mammals, and Hb represents approximately 65% of the Fe found in the body (Muñoz et al., 2009). Hemoglobin serves as a transport protein that carries oxygen through the bloodstream from the lungs to tissues. In young swine, inefficient absorption of Fe reduces the number of circulating red blood cells resulting in Fe deficiency and poor growth performance (Kim et al., 2018). Dietary nonheme Fe is found primarily in the Fe^{2+} or ferric (Fe^{3+}) forms. The Fe^{3+} form of Fe has low solubility in the stomach, which decreases its availability compared with that of the Fe^{2+} form (Fuqua et al., 2012). The DMT-1 transport protein is the main facilitator of the Fe^{2+} form of Fe entry into enterocytes of the duodenum (Conrad and Umbreit, 2002). Once transported to the duodenum, Fe is transferred across the duodenum and transported via ferroportin to red blood cells or bone marrow for erythropoiesis (Xue and Shah, 2013).

Rincker et al. (2004) observed increases in ADG and Hb and Hct values with increasing Fe supplementation from $FeSO_4$ up to 150 mg/kg of the diet. Moreover, Lee et al. (2008) observed similar results where increasing $FeSO_4$ up to 250 mg/kg of the diet increased Hb values in postweaned pigs. These results suggest that the Fe requirement to optimize growth and hematological criteria is likely above 100 mg/kg of the diet and that the Fe provided in common feed ingredients will not necessarily meet

these requirement estimates. Thus, supplemental dietary Fe sources should be added to nursery diets to meet postweaning Fe requirements for growth and blood Fe status. In our study, we purposely did not provide injectable Fe after birth and fed deficient Fe concentrations in the diet in order to increase the sensitivity of our bioassay.

Research has been conducted to understand if different forms of Fe are more readily absorbed or have increased bioavailability compared with $FeSO_4$. Ettle et al. (2008) observed an improvement in ADG and G:F and increased Hb and Hct values when increasing levels of either Fe-glycinate or $FeSO_4$ were supplemented in nursery diets. Feng et al. (2007) observed no evidence of difference in Hb concentration of nursery pigs when adding 120 mg/kg of Fe from either an Fe-glycine chelate or $FeSO_4$. Novais et al. (2016) observed no evidence for a difference in growth performance or hematological criteria in nursery pigs when adding 67.5 mg/kg of Fe from $FeSO_4$ or 150 mg/kg from a proteinated Fe. These studies suggest that other Fe sources in addition to $FeSO_4$ are effective at optimizing postweaning growth performance and blood Fe status. The study herein indicates that the $FeCO_3$ source used is an alternate source of dietary Fe that is as equally effective as $FeSO_4$ to support postweaning growth performance and blood Fe status similar to previous studies testing other Fe sources.

Iron sulfate and $FeCO_3$ are both nonheme dietary sources of Fe found in the Fe^{2+} form, which would suggest that both sources should utilize the same pathway for uptake by the duodenum and have similar bioavailability. Limited research is available on the effects of $FeCO_3$ on nursery pig growth performance and hematological criteria. Ammerman et al. (1974) conducted two separate experiments in nursery pigs evaluating different feed-grade $FeCO_3$ sources compared with $FeSO_4$ and observed that, in both experiments, $FeSO_4$ provided the greatest improvements in ADG, Hb concentration, and Hct levels. The results of the study herein would disagree with the observations of Ammerman et al. (1974). The $FeCO_3$ source used in the study herein potentially has improved the bioavailability compared with that of the older sources used by Ammerman et al. (1974). The improvement in bioavailability potentially could be due to the processes involved in the manufacturing of the $FeCO_3$ source used in our study. Feed grade ferrous carbonate is processed via wet milling, resulting in a very small particle size. Then, the next step in the process is to spray-dry the material on to a digestible binder and a solvent to form an agglomerated finished product. This potentially leads to improved digestion and uptake across the duodenal membrane.

A limitation of the current study would be the low number of replications (pens) per treatment. This was done intentionally to limit the number of pigs needed for the study as they did not receive an Fe injection at birth as is standard in the swine industry. Indeed, the pigs in the current study would be classified as anemic if Hb concentrations at the start of feeding experimental diets were below 10 g/dL (Bhattarai and Nielsen, 2015). Hemoglobin regeneration has been used in Fe depletion–repletion studies to measure the bioavailability of Fe sources compared with that of $FeSO_4$ (Chausow and Czarnecki-Maulden, 1988; Biehl et al., 1997). In the study herein, a day effect was observed as Hb concentrations were increased throughout the study. This demonstrates that regeneration of Hb occurred and pigs improved their blood Fe status with normal feeding behaviors. Because there was no evidence for a difference between $FeSO_4$ or $FeCO_3$ in Hb concentration at any of the collection time points, this implies $FeCO_3$ is a suitable source for Fe fortification in nursery diets.

In summary, this study has provided evidence that providing either $FeSO_4$ or $FeCO_3$ in diets fed to pigs that are Fe-deficient elicited similar improvements in nursery pig growth performance and hematological status. Our study also demonstrated that the Fe-deficient model utilized was sufficient for evaluating the availability of the two different Fe sources and their effects on nursery pig growth performance and hematological criteria. Based on these observations, the data suggest that this micronized form of $FeCO_3$ is a sufficient alternative source of dietary Fe that can be added to nursery diets to meet postweaning requirements.

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Conflict of interest statement

The authors declare no conflict of interest; however, R.S.F., M.E.K., and J.L.U. are employees of Micronutrients USA, LLC, Indianapolis, IN, the company that provided partial financial support.

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