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A cooperative study assessing the effects of a second iron injection administered before weaning on growth performance, hematological status, and tissue mineral concentrations of nursery pigs*

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ABSTRACT

Objective: A study involving 7 experiment stations evaluated the effects of a second iron injection administered before weaning on growth and hematological measures of pigs.

Materials and Methods: Pigs (n = 514) were given an iron injection (100–200 mg) on the first day of life. Piglets were then allotted to pairs of similar-weight, same-sex siblings 3 to 5 d before weaning (on d 18–24) with one piglet from each pair receiving a second iron injection. All pigs received common station-specific postweaning diets. Data were subjected to ANOVA with the model containing the terms treatment, station, pair within station, and treatment × station interaction.

Results and Discussion: Postweaning ADG was greater for the added-injection group during during 0 to 14 d after weaning, but the response (212.5 vs. 202.6 g) was largely influenced by a single station as evidenced by a treatment × station interaction. The tendency for a treatment × station interaction for overall ADG (d −4 to 28) indicated that iron status was not the most limiting factor for growth at all stations. Hemoglobin concentration was greater for the added-injection group at weaning and d 14 after weaning.

Implications and Applications: An additional iron injection before weaning may lead to improved early nursery growth; however, the beneficial effects of an additional iron injection are not universal and are likely dependent on unique herd characteristics including timing and total dosage of iron injections as well as nursery diet supplementation.

Key words: growth, hemoglobin, injection, iron, piglets

INTRODUCTION

It is routine practice in swine production to supplement 100 to 200 mg of iron via i.m. injection to newborn pigs shortly after birth as they have limited stores of iron (Venn et al., 1947). Moreover, it has been suggested that the iron requirement of piglets during lactation is mainly dependent on the growth rate of the piglet during the lactation period (Venn et al., 1947; Kamphues et al., 1992; Egeli and Framstad, 1998; Van Gorp et al., 2012). Modern swine production has undergone improvements in genetics that have resulted in increased litter sizes and growth rates of nursing piglets. Given these advancements, recent work has shown that the industry standard iron injection (100–200 mg of Fe) administered to piglets shortly after birth is not sufficient to maintain the iron status of the pig throughout the entire lactation period (Bhattarai and Nielsen, 2015a; Perri et al., 2016). In addition, it has been observed that the faster-growing pigs in a litter are most susceptible to becoming iron deficient at weaning, creating potential postweaning problems or limitations (Jolliff and Mahan, 2011).

Recently, Chevalier et al. (2021) reported that pigs administered 200 mg of iron at birth had peak hemoglobin (**Hb**) concentration on d 17 of age that subsequently de-

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clined to weaning at d 22. Furthermore, pigs that only received 100 mg of iron at birth had peak Hb at d 11 of age with a decline in Hb thereafter. These results are in agreement with previous literature that suggests a possibility of pigs having suboptimal hemoglobin concentrations (generally <11 g/dL as defined by Thorn, 2010; Bhattarai and Nielsen, 2015b; Perri et al., 2016) at weaning. Interestingly, it has been demonstrated that pigs with optimal hemoglobin concentrations at weaning have greater growth performance in the subsequent postweaning periods (Bhattarai and Nielsen, 2015b; Fredericks et al., 2018; Chevalier et al., 2023). Furthermore, Olsen (2020) demonstrated that an additional iron injection given to piglets 4 to 6 d after the initial iron injection resulted in a much larger percentage (89% vs. 20%) of pigs having optimal Hb concentration (≥11 g/dL) at weaning and a 454-g increase in BW 42 d after weaning. Thus, although it seems that there may be a potential benefit to administration of an additional iron injection before weaning, it is not known whether that response would be a universal response across different production systems. Thus, the objective of this experiment was to evaluate the effects of administering an additional iron injection 3 to 5 d before weaning on pig growth performance, hematological status, and tissue mineral concentration before and after weaning across multiple locations with varied management practices.

MATERIALS AND METHODS

Animals and Experimental Design

A cooperative study using 514 pigs was conducted at 7 universities to evaluate the effects of a second iron injection administered to pigs before weaning on pre- and postweaning growth performance, hematological measures, and tissue mineral content. Participating universities included Oklahoma State University, Purdue University, South Dakota State University, University of Arkansas, University of Georgia, University of Kentucky, and Virginia Tech. Research at individual stations followed the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2020) and was conducted under protocols approved by the respective Institutional Animal Care and Use Committees.

Individual characteristics (number of animals, processing age, iron dosage, weaning age, and so on) of the participating research stations are described in Table 1. Stations are represented by an assigned number to maintain confidentiality. Each station contributed to growth performance data but fewer stations to blood and tissue sampling. Data contributions by station can be found in Table 1. All pigs used in this experiment were administered an initial i.m. iron injection (100 to 200 mg of Fe from Fe dextran) at postfarrowing litter processing, with the dosage being station specific (Table 1). At each station, pigs were assigned to either the control (**CON**) or an added-injection treatment (**+Fe**) by pairing 2 same-sex siblings with a BW difference ≤0.453 kg at the preweaning injection time. One pig within each pair received the additional iron injection (same dose received at processing) 3 to 5 d before weaning. No creep feed was offered during lactation, but access to the sow's feed was not restricted. Pigs were weaned (18 to 24 d of age) and placed in pens with pigs from the same treatment. Once weaned, both the control and added-injection group received common station-specific nursery diets. Nursery diets fed to pigs at all the stations were based on corn and soybeans and met or exceeded NASEM (2012) nutrient-requirement estimates for the weight of the pigs being fed. Because both the CON and +Fe pigs at a particular station received the same nursery diet and given there were many differences in ingredients used at these stations, diet compositions were reviewed but are not provided. Some stations fed 4 diets across the 4-wk period (with initial diets for only 2 or 3 d in some cases), whereas other stations fed 2 or 3 diets, reflecting a wide diversity of feeding management. However, it is worth noting that stations 2, 3, and 7 supplied zinc oxide at inclusion rates greater than 1,500 mg/kg Zn. In addition, stations 2 and 6 provided phytase to the diets. Other dietary ingredients such as animal by-products can provide additional dietary iron, and those were included for varying diets and periods of time at different stations. Thus, it is also worth noting that stations 1, 2, 3, 4, and 7 included fish meal (0% to 8% inclusion); stations 1, 2, and 7 included plasma protein (0% to 5% inclusion); and stations 2 and 7 included spray-dried blood meal (0% to 6.5% inclusion). Analyzed Fe, Cu, and Zn concentrations of nursery diets fed at the 3 stations that participated in blood hematology measures are presented in Table 2; regarding the analyzed Fe concentration, when comparing the supplemented Fe to the analyzed Fe at those 3 stations, the analyzed values in all instances were from 120 to 160 mg/kg above the supplemented level from the trace mineral premix. Pigs were provided *ad libitum* access to the diets.

Measurements and Sample Collection

Individual pig BW was recorded weekly for 4 wk by all 7 stations. Blood samples were also collected by 3 of the 7 stations at d −4 (second injection) and 0 (weaning) and at d 14 and 28 after weaning. Blood samples were collected via vena cava puncture into EDTA-containing tubes (BD Corporate, Franklin Lakes, NJ). Samples were later analyzed for a complete blood count using a hematological analyzer (Forcyte Veterinary Hematology Analyzer, Oxford Science, Oxford, CT, at station 1; Abbott Cell Dyn 3500 Veterinary Hematology Analyzer, Abbott Diagnostics, Santa Clara, CA, at station 3; and Hemavet FS950, Drew Scientific, Miami Lakes, FL, at station 6) according to the manufacturer's directions. All 3 stations analyzed blood for Hb, hematocrit (**HCT**), red blood cell count (**RBC**), and white blood cell count (**WBC**). Two stations

Table 1. Distinctive characteristics of stations involved in the study

	Experiment station						
Item	1	2	3	4	5	6	7
Pairs of pigs used, no.	59	13	70	37	18	39	21
Breed of pigs[1]	W sire on YL females	D sire on YL females	D sire on YLW females	D sire on YL females	Various CG sire on CG Line CG32 females	DNA600 sire (Duroc) on PIC 29 females	W sire on YL females
Time of injection							
First injection, h after farrowing	<24	<24	<24	<24	<24	<24	<24
Second injection, d before weaning	−4	−4	−4	−3, −4	−4	−5	−5
Weaning age, d	18–24	19–21	17–21	18–24	18–20	19–21	18
Iron injection amount, mg	150	100	200	100	200	100	150
Supplemental Fe in nursery diet, mg/kg	100	121	346	145	100	266	91
Data collected[2]							
Growth performance	X	X	X	X	X	X	X
Diet samples	X	X	X	—	—	X	X
Hematology	X	—	X	—	—	X	—
Tissue	X	—	—	—	—	—	—

[1]L = Landrace, Y = Yorkshire, W = Large White, D = Duroc, CG = Choice Genetics, PIC = Pig Improvement Company.

[2]An "X" represents stations that supplied data for that particular response line, whereas a dash represents stations that did not supply data.

also analyzed blood for mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), and mean corpuscular hemoglobin concentration (**MCHC**), and one station further analyzed blood for red cell distribution width, platelet count, mean platelet volume, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Additionally, one station also took tissue samples (liver, spleen, heart, and kidney) for tissue mineral content. Tissue samples were collected from 4 barrows per treatment at the same time of blood sampling (d −4, 0, 14, and 28). All tissues were ground and mixed to a homogeneous mixture, and a subsample was digested by a microwave digestion oven (MARS 6; CEM Corporation, Matthews, NC) using nitric acid and procedures recommended by the manufacturer. After digestion, tissue digests were appropriately diluted and analyzed for trace mineral content (Fe, Zn, and Cu) using flame atomic absorption spectrophotometry (Thermoelemental, SOLAAR Mf; Thermo Electron Corp., Verona, WI).

Statistical Analysis

Growth performance and hematological data were analyzed by ANOVA for a randomized complete block design using PROC GLM of SAS (version 9.4, SAS Institute Inc., Cary, NC). Data were initially evaluated for statistical outliers within each station using the Grubb's test outlier calculator (GraphPad Software, San Diego, CA), but no outliers were detected. All data were then tested for sex effects and treatment × sex interactions; however, these model terms were not significant. The final model included treatment, station, pair nested within station, and treatment × station interaction, with the pig being the experimental unit. For the blood and tissue response measures that were provided by only one station, the station

Table 2. Analyzed mineral composition of nursery diets fed at stations that participated in blood sampling[1]

	Experiment station		
Item	1	3[2]	6
d 0 to 14			
Fe	220.3	481.9	432.7
Zn	138.4	2,153.3	149.5
Cu	18.9	166.7	9.4
d 14 to 28			
Fe	246.6	444.6	404.1
Zn	139.5	1,871.6	184.6
Cu	18.5	153.3	14.5

[1]Analyzed mineral concentrations are presented as milligrams per kilogram of diet.

[2]Station 3 supplied ZnO in the nursery diets.

Table 3. Effects of an additional iron injection administered before weaning on growth performance of pigs at 7 experimental stations[1]

Item	TRT[2] CON	TRT[2] +Fe	SEM	*P*-value TRT	*P*-value Station	*P*-value TRT × station
BW, kg						
d −4	5.18	5.18	0.01	0.75	<0.001	0.63
d 0	6.20	6.21	0.02	0.85	<0.001	0.46
d 7	7.02	7.07	0.03	0.33	<0.001	0.55
d 14	9.04	9.18	0.05	0.05	<0.001	0.14
d 21	12.33	12.43	0.08	0.37	<0.001	0.07
d 28	15.86	16.00	0.11	0.35	<0.001	0.07
ADG, g						
d −4 to 0	246.00	248.36	4.35	0.70	<0.001	0.14
d 0 to 7	117.66	122.94	3.43	0.28	<0.001	0.20
d 7 to 14	289.37	304.51	4.79	0.03	<0.001	0.04
d 14 to 21	457.75	451.09	6.30	0.46	<0.001	0.06
d 21 to 28	496.92	502.80	6.91	0.55	<0.001	0.08
d −4 to 28	331.41	335.82	3.17	0.33	<0.001	0.09

[1]Data represent 514 pigs; d 21 and 28 data represent 506 pigs. All pigs received an initial iron injection at processing of the litter following birth; the added group received an additional iron injection 3 to 5 d before weaning (d 0). Individual station responses are provided in Table 4.

[2]TRT = treatment; CON = control group; +Fe = the added-injection group.

term was dropped from the model. All data are reported as LSM, with statistical differences being considered significant at $P \leq 0.05$ and a tendency for significance at $P \leq 0.10$.

RESULTS AND DISCUSSION

A total of 514 pigs from 7 experiment stations were used in this investigation. At weaning the sample size was reduced to 506 pigs because a subset of pigs was used for tissue mineral content at one station.

Growth Performance

There was a station effect observed every week for both BW and ADG (Table 3; the station effects can be further evaluated in Table 4). These findings were expected and are indicative of differences such as genetics, management, nursery environment, diet, and so on among stations. With regard to the iron injection, ADG was greater for the +Fe group for d 7 to 14 after weaning (Table 3; $P = 0.03$). The improved ADG observed resulted in a greater BW for the +Fe pigs on d 14 ($P = 0.05$). In agreement, Kamphues et al. (1992) reported that pigs administered a second iron injection 1 wk before weaning had an increase in daily BW gain (380 vs. 362 g) through 3 wk in the nursery. However, in the present study that increase was associated with a treatment × station interaction ($P = 0.04$) and was not observed universally. There were also tendencies for a treatment × station interaction for ADG for d 14 to 21 and d 21 to 28 after weaning. Although there was not a treatment effect for overall ADG (d −4 to 28 after weaning), the tendency ($P = 0.09$) for a treatment × station interaction that was initially observed beginning in the second week remained for the entire study period. The tendency for a treatment × station interaction (Table 3, $P = 0.09$) for overall ADG (d −4 to 28 after weaning) illustrated both responsive and nonresponsive stations. Table 4 presents the treatment responses by station for growth performance. Although 6 of 7 stations observed a numerical increase in ADG for d 0 to 14 after weaning (with an overall treatment improvement of 212.5 vs. 202.6 g/d, $P = 0.03$), only for 2 stations was it significant ($P < 0.05$). Pigs at only one station responded with a positive increase in ADG for d 14 to 28 after weaning and d −4 to 28 after weaning, clearly indicating that iron status was not the most limiting factor for growth at all stations.

Some recent work suggests that an additional iron injection administered before weaning can lead to heavier nursery weights (Olsen, 2020). Additionally, in a more recent study where pigs were tracked through slaughter, Chevalier et al. (2023) reported that pigs receiving a second iron injection on d 6 to 8 of lactation resulted in a 4% increase in ADG from weaning to slaughter, resulting in a ~3-kg heavier pig at slaughter. However, similar to the nonresponsive experiment stations herein, Williams et al. (2020) found no effect on growth performance in pigs given a second iron injection. Notably, however, Williams et al. (2020) administered the second iron injection on d

Table 4. Effects of an additional iron injection administered before weaning on growth performance stratified by experiment station[1]

Station, TRT[2]	BW, kg				ADG, g			
	d −4	d 0	d 14	d 28	d −4 to 0	d 0 to 14	d 14 to 28[3]	d −4 to 28
1								
CON	5.41^{c}	6.47^{c}	11.46^{b}	21.63^{b}	265.2^{c}	356.9^{b}	636.1^{b}	497.8^{b}
+Fe	5.44^{c}	6.48^{c}	11.92^{a}	22.59^{a}	260.4^{c}	388.6^{a}	666.0^{a}	525.5^{a}
2								
CON	5.71^{b}	6.77^{b}	10.82^{c}	18.07^{c}	265.1^{c}	289.3^{c}	517.5^{d}	386.1^{cd}
+Fe	5.69^{b}	6.81^{b}	10.99^{c}	17.53^{c}	279.9^{c}	298.7^{c}	466.7^{de}	369.9^{d}
3								
CON	4.83^{d}	6.30^{d}	9.85^{d}	17.85^{c}	367.1^{a}	253.8^{d}	571.3^{c}	407.0^{c}
+Fe	4.82^{d}	6.17^{e}	9.82^{d}	17.75^{c}	337.7^{b}	260.4^{d}	566.6^{c}	403.9^{c}
4								
CON	6.41^{a}	7.38^{a}	8.81^{e}	15.40^{d}	272.5^{c}	101.9^{f}	471.0^{de}	285.0^{gf}
+Fe	6.35^{a}	7.36^{a}	9.00^{e}	15.63^{d}	279.5^{c}	117.3^{f}	473.4^{de}	294.0^{ef}
5								
CON	4.52^{e}	5.30^{g}	8.08^{f}	12.57^{f}	195.6^{de}	198.7^{e}	320.5^{g}	251.5^{h}
+Fe	4.49^{e}	5.36^{g}	7.93^{f}	12.90^{f}	215.4^{d}	183.9^{e}	354.9^{fg}	262.6^{gh}
6								
CON	4.85^{d}	5.78^{f}	6.06^{g}	11.31^{g}	185.9^{de}	19.8^{h}	371.1^{f}	189.8^{i}
+Fe	4.84^{d}	5.79^{f}	6.35^{g}	11.21^{g}	190.1^{de}	39.9^{g}	347.3^{fg}	187.4^{i}
7								
CON	4.54^{e}	5.38^{g}	8.12^{f}	14.27^{e}	169.3^{e}	195.6^{e}	439.0^{e}	304.1^{ef}
+Fe	4.58^{e}	5.45^{g}	8.25^{f}	14.46^{e}	175.35^{e}	199.9^{e}	443.6^{e}	308.9^{e}
SEM	0.01	0.02	0.05	0.11	4.35	3.28	5.20	3.17

[a–i]Means within a column with different superscripts differ ($P < 0.05$).

[1]Data represent 514 pigs; d 14 and 28 data represent 506 pigs. All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 3 to 5 d before weaning (d 0).

[2]TRT = treatment; CON = control group; +Fe = the added-injection group.

[3]TRT × station interaction ($P = 0.05$).

11 of age and weaned pigs on d 21 of age compared with the current experiment where the second injection was administered at 13 to 20 d of age (d −3 to −5 before weaning). Interestingly, the work by Olsen (2020) reported that pigs were administered an additional iron injection around d 6 to 10 of age and weaned around 25 d of age. Thus, the time of the second injection and the weaning age may be critical. Additionally, there may be other factors such as genetics, herd health status, or environmental conditions that are more limiting, which would further explain the growth benefit observed in some locations but not others. Similar to station differences in the current experiment, there are many differences between swine production farms in the current swine industry. As a result, it may be appropriate to evaluate iron status on an individual farm or herd basis to determine whether that farm or herd might respond with a positive growth response.

It is thought that optimizing the hemoglobin concentration and the overall iron status of pigs can promote maximum immunity, thereby increasing the health status of pigs (Perri et al., 2016). Optimizing health status before weaning can be a major contributor to subsequent growth performance in the nursery as this transition can be very stressful for young pigs. Work conducted by Fredericks et al. (2018) revealed that pigs with optimal hemoglobin status (>11 g/dL) at weaning had a greater BW at 8 wk after weaning in contrast to pigs with lower hemoglobin concentrations (<11 g/dL). In the present experiment, pigs administered the second iron injection 3 to 5 d before weaning had a numerically heavier final BW at 4 wk in the nursery and had a mean Hb concentration above the optimal level at weaning, agreeing with the previous literature (Haugegaard et al., 2008; Jolliff and Mahan, 2011; Olsen, 2020, Williams et al., 2020). The difference in final BW between treatments would be a function of the accumulation of increased ADG in conjunction with the length of the experiment for the treatment pigs; thus, some differences between published studies would be a function of the experimental procedures (i.e., the length of a given study).

Hematological Status

Pigs from 3 of the 7 research stations were used to measure hematological status at d −4, 0, 14, and 28 rela-

Table 5. Effects of an additional iron injection on white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (Hb), and hematocrit content (HCT)[1,2]

	TRT[3]			*P*-value		
Item	CON	+Fe	SEM	TRT	Station	TRT × station
d −4[4]						
WBC, ×10³/μL	7.2	7.2	0.20	0.80	<0.001	0.58
RBC, ×10⁶/μL	5.6	5.5	0.06	0.25	0.03	0.21
Hb, g/dL	10.3	10.1	0.11	0.29	<0.001	0.77
HCT, %	32.9	32.3	0.36	0.22	<0.01	0.71
d 0[4]						
WBC, ×10³/μL	7.6	9.1	0.27	<0.001	0.73	0.91
RBC, ×10⁶/μL	5.9	6.1	0.06	0.02	0.16	0.22
Hb, g/dL	9.8	11.1	0.11	<0.001	<0.001	0.09
HCT, %	32.1	36.2	0.36	<0.001	0.13	0.23
d 14[5]						
WBC, ×10³/μL	16.5	16.7	0.40	0.78	<0.001	0.81
RBC, ×10⁶/μL	7.0	7.0	0.07	0.69	<0.001	0.37
Hb, g/dL	11.1	11.9	0.11	<0.001	<0.01	<0.01
HCT, %	35.5	37.1	0.34	0.001	<0.01	0.01
d 28[6]						
WBC, ×10³/μL	15.3	14.4	0.42	0.14	<0.001	0.51
RBC, ×10⁶/μL	6.8	6.8	0.08	0.76	<0.01	0.46
Hb, g/dL	11.2	11.4	0.11	0.25	<0.001	0.20
HCT, %	36.1	36.4	0.38	0.48	<0.001	0.48

[1]These hematology measures were taken at 3 of the 7 stations. All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 4 to 5 d before weaning (d 0).

[2]Merck (2016) reference ranges for WBC, RBC, Hb, and HCT, for pigs in general without respect to age or health status, are 11 to 22, 5 to 8, 10 to 16, and 36 to 43, respectively.

[3]TRT = treatment; CON = control group; +Fe = the added-injection group.

[4]Means represent 104 pigs per treatment.

[5]Means represent 82 pigs per treatment.

[6]Means represent 77 pigs per treatment.

tive to weaning. Table 5 presents the WBC, RBC, Hb, and HCT data using the individual pig as the experimental unit. Pigs from both the CON and +Fe groups had similar WBC, RBC, Hb, and HCT profiles at the d −4 sampling ($P > 0.10$). However, at weaning, the +Fe group had greater ($P \leq 0.02$) WBC, RBC, Hb, and HCT values. The improvement in hematological measures at weaning in the current experiment was expected. These findings are in agreement with other work in which a second iron injection improved hematological measures (Haugegaard et al., 2008; Williams et al., 2020; Chevalier et al., 2023).

Interestingly, at weaning the CON group had lower Hb and HCT values than the previous sampling at d −4, whereas the +Fe group was elevated. Holter et al. (1991) and Jolliff and Mahan (2011) reported a reduction in hematological measures as early as 17 d of age for pigs that were administered an iron supplement at birth (180 and 200 mg of iron). Furthermore, recently published work (Chevalier et al., 2021) looking at the hematological profile in pigs throughout lactation and after weaning after receiving increasing iron doses revealed that Hb began to decrease starting at d 11 of age for pigs receiving a 100-mg Fe injection at birth and at d 17 of age for pigs receiving a 200-mg Fe injection at birth. Considering these observations with regard to a standard weaning age in the United States of ~21 d and even older in other countries, it seems that administering a second iron injection at least 4 d before weaning may warrant consideration as an appropriate intervention method for herds with lower hematological status at weaning.

Hemoglobin concentration and HCT continued to be greater ($P = 0.001$) in the +Fe group at d 14 after weaning but not at d 28 after weaning. In a similar study (Chevalier et al., 2023), pigs administered a second iron injection before weaning had greater Hb levels compared with the control pigs ($P = 0.01$) at 35 d after weaning, but no differences in Hb were observed at slaughter (~147 d).

Table 6. Station effects of an additional iron injection on white blood cell (WBC) and red blood cell (RBC) counts[1]

Station, TRT[2]	WBC, ×10³/μL				RBC, ×10⁶/μL			
	d −4	d 0	d 14	d 28	d −4	d 0	d 14	d 28
1								
CON	8.0[a]	7.7[b]	14.4[b]	13.2[b]	5.6[a]	5.9[b]	6.4[b]	6.7[ab]
+Fe	8.0[a]	9.3[a]	14.7[a]	12.7[b]	5.7[a]	6.2[a]	6.3[b]	6.6[a]
3								
CON	6.8[b]	7.5[b]	—	—	5.5[ab]	5.8[b]	—	—
+Fe	6.3[b]	9.3[a]	—	—	5.4[ab]	5.9[b]	—	—
6								
CON	7.0[b]	7.5[b]	18.6[a]	17.3[a]	5.6[ab]	6.0[ab]	7.6[a]	6.9[a]
+Fe	7.3[ab]	8.8[ab]	18.7[a]	16.0[a]	5.3[b]	6.2[ab]	7.6[a]	7.00[a]
SEM	0.20	0.27	0.40	0.42	0.06	0.06	0.07	0.08

[a,b]Means within a column with different superscripts differ ($P < 0.05$).

[1]All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 4 to 5 d before weaning (d 0). Station 3 did not blood sample on d 14 and 28; therefore, a dash is in place for those data points.

[2]TRT = treatment; CON = control group; +Fe = the added-injection group.

There was a tendency ($P = 0.09$) for a treatment × station interaction at d 0 for Hb, which was more pronounced ($P < 0.01$) at d 14 after weaning. There was also a treatment × station interaction observed for HCT at d 14 after weaning ($P = 0.01$). The station effects and treatment × station interactions are provided in Tables 6 and 7. These interactions likely derive from the different amounts of iron supplemented by the different stations. It is worth noting that the 3 stations that participated in hematological measures all administered different amounts of injectable iron dextran as well as supplied different amounts of iron in the nursery diet. With regard to these differences,

Table 7. Station effects of an additional iron injection administered before weaning on hemoglobin concentration (Hb) and hematocrit content (HCT)[1]

Station, TRT[2]	Hb, g/dL				HCT, %			
	d −4	d 0[3]	d 14[4]	d 28	d −4	d 0	d 14[5]	d 28
1								
CON	10.9[a]	10.4[c]	11.5[a]	12.8[a]	33.1[ab]	31.5[c]	35.2[b]	38.1[a]
+Fe	10.7[a]	12.0[a]	11.9[ac]	12.8[a]	32.8[ab]	36.5[a]	35.6[b]	38.1[a]
3								
CON	10.6[a]	10.4[c]	—	—	33.9[a]	33.4[b]	—	—
+Fe	10.6[a]	11.3[b]	—	—	33.5[ab]	36.4[a]	—	—
6								
CON	9.3[b]	8.8[d]	10.6[b]	9.6[b]	31.8[bc]	31.4[c]	35.9[b]	34.0[b]
+Fe	8.9[b]	9.9[c]	11.9[ac]	10.0[b]	30.6[c]	35.6[a]	38.6[a]	34.7[b]
SEM	0.11	0.11	0.11	0.11	0.36	0.36	0.34	0.38

[a–d]Means within a column with different superscripts differ ($P < 0.05$).

[1]All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 4 to 5 d before weaning (d 0). Station 3 did not blood sample on d 14 and 28; therefore, a dash is in place for those data points.

[2]TRT = treatment; CON = control group; +Fe = the added-injection group.

[3]TRT × station interaction ($P = 0.09$).

[4]TRT × station interaction ($P < 0.01$).

[5]TRT × station interaction ($P = 0.01$).

Table 8. Effects of an additional iron injection on mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)[1,2]

	TRT[3]			*P*-value		
Item	CON	+Fe	SEM	TRT	Station	TRT × station
d −4[4]						
MCV, fL	57.9	57.9	0.42	0.92	0.22	0.10
MCH, pg	18.0	17.9	0.16	0.73	<0.001	0.09
MCHC, g/dL	31.0	31.0	0.12	0.79	<0.001	0.39
d 0[4]						
MCV, fL	53.2	58.3	0.38	<0.001	0.04	0.64
MCH, pg	16.2	17.7	0.14	<0.001	<0.001	0.80
MCHC, g/dL	30.4	30.4	0.13	0.77	<0.001	0.77
d 14[5]						
MCV, fL	51.1	53.7	0.32	<0.001	<0.001	0.08
MCH, pg	16.0	17.3	0.12	<0.001	<0.001	0.03
MCHC, g/dL	31.2	32.1	0.14	<0.001	<0.001	0.05
d 28[5]						
MCV, fL	52.9	53.7	0.32	0.07	<0.001	0.84
MCH, pg	16.5	16.9	0.13	0.05	<0.001	0.64
MCHC, g/dL	31.0	31.2	0.18	0.41	<0.001	0.32

[1]These hematology measures were taken at 2 of the 7 stations. All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 4 to 5 d before weaning (d 0).

[2]Merck (2016) reference ranges for MCV, MCH, and MCHC, for pigs in general without respect to age or health status, are 50 to 68, 17 to 21, and 30 to 34, respectively.

[3]TRT = treatment; CON = control group; +Fe = the added-injection group.

[4]Means represent 82 pigs per treatment.

[5]Means represent 77 pigs per treatment.

station 3 administered the greatest dose of iron but did not have the greatest response; they also had the greatest amount of supplemental iron in the nursery diet. The largest numerical response was actually observed at station 1, which administered the intermediate dose of supplemental iron for those 3 stations. Thus, it is evident that hematological status, and the growth response associated with that status, is a multifactorial issue and not simply an issue of the dose of iron injected or whether there is a second injection.

Similar to previous hematological measures, Table 8 demonstrates no differences between the CON and +Fe groups with respect to MCV, MCH, and MCHC profiles at d −4. However, by weaning, MCV and MCH values were greater ($P < 0.001$) in the +Fe pigs, which remained greater at d 14 after weaning when MCHC is also greater ($P < 0.001$) in the +Fe pigs. Furthermore, for the +Fe group at d 28 after weaning, MCH remained greater ($P < 0.05$) and MCV tended to be greater ($P = 0.07$), but no difference for MCHC was observed compared with the CON group. Mean corpuscular volume, MCH, and MCHC had station effects at all sampling time points with the exception of d −4 for MCV. At d 14 after weaning, there was a tendency for a treatment × station interaction for MCV ($P = 0.08$), whereas a more pronounced interaction was observed for MCH and MCHC ($P = 0.03$ and $P = 0.05$, respectively); the tendency was one of magnitude wherein station 1 had a greater response to the +Fe treatment for MCV, MCH, and MCHC compared with station 6 (data not shown).

Tables 9 and 10 represent data collected by only one research station. There was a tendency ($P = 0.06$) for eosinophil count to be greater in the +Fe group at weaning compared with the CON group (Table 9), which may explain the increase in WBC at weaning that was reported in Table 5. However, there were no other effects observed on leukocyte count during the experiment. Red cell distribution width, platelet count, and mean platelet volume (Table 10) were all similar between the CON and +Fe groups at d −4, but at weaning red cell distribution width was less in the +Fe group compared with the CON group. Red cell distribution width remained less ($P \leq 0.01$) in the +Fe pigs at d 14 and 28 after weaning when compared with the CON pigs. The +Fe group had a greater mean platelet volume at d 14 after weaning ($P < 0.001$), and on d 28 after weaning, the +Fe group had a tendency to have a lesser platelet count ($P = 0.06$), compared with the CON group.

Table 9. Effects of an additional iron injection on leukocyte count (%) of pigs from station 6[1,2]

	TRT[3]			
Item	CON	+Fe	SEM	*P*-value
d −4				
Neutrophils	33.4	37.0	1.82	0.18
Lymphocytes	58.0	55.6	1.98	0.39
Monocytes	3.5	3.3	0.27	0.69
Eosinophils	4.8	3.7	0.50	0.12
Basophils	0.24	0.43	0.09	0.15
d 0				
Neutrophils	43.1	42.7	2.41	0.90
Lymphocytes	48.2	46.6	2.38	0.63
Monocytes	3.1	3.5	0.30	0.40
Eosinophils	5.3	7.0	0.59	0.06
Basophils	0.22	0.26	0.06	0.62
d 14				
Neutrophils	38.3	36.1	1.39	0.27
Lymphocytes	32.9	31.4	1.14	0.35
Monocytes	5.7	6.4	0.30	0.15
Eosinophils	22.3	25.3	1.49	0.17
Basophils	0.70	0.83	0.12	0.43
d 28				
Neutrophils	32.0	32.8	0.94	0.55
Lymphocytes	37.0	37.6	1.79	0.79
Monocytes	7.5	7.2	0.58	0.72
Eosinophils	22.4	21.2	1.36	0.53
Basophils	1.03	1.08	0.16	0.82

[1]These hematology measures were taken at only 1 of the 7 stations. All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 5 d before weaning (d 0). Means represent 22 pigs per treatment.

[2]Merck (2016) reference ranges for neutrophils, lymphocytes, monocytes, eosinophils, and basophils, for pigs in general without respect to age or health status, are 28 to 47, 39 to 62, 2 to 10, 0.5 to 11, and 0 to 2, respectively.

[3]TRT = treatment; CON = control group; +Fe = the added-injection group.

Table 10. Effects of an additional iron injection on red cell distribution width (RDW), platelet count (PLT), and mean platelet volume (MPV) of pigs from station 6[1,2]

	TRT[3]			
Item	CON	+Fe	SEM	*P*-value
d −4				
RDW, %	27.2	27.2	0.67	0.99
PLT, ×10³/µL	524.3	500.9	39.17	0.68
MPV, fL	10.6	10.5	0.27	0.77
d 0				
RDW, %	31.2	27.5	0.65	<0.01
PLT, ×10³/µL	584.1	573.8	36.25	0.84
MPV, fL	10.1	10.5	0.18	0.12
d 14				
RDW, %	30.7	27.0	0.57	<0.001
PLT, ×10³/µL	563.7	538.4	41.37	0.67
MPV, fL	9.5	10.9	0.26	<0.01
d 28				
RDW, %	27.3	25.2	0.40	<0.01
PLT, ×10³/µL	542.1	470.2	26.49	0.06
MPV, fL	9.8	10.3	0.27	0.19

[1]All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 5 d before weaning (d 0). Means represent 22 pigs per treatment.

[2]Merck (2016) reference range for platelet count, for pigs in general without respect to age or health status, is 200 to 500.

[3]TRT = treatment; CON = control group; +Fe = the added-injection group.

Integrating the growth data (Table 4) for stations 1, 3, and 6 with some of the blood data collected (Tables 6 and 7) demonstrates that improvements in ADG are not simply a function of improved blood responses. Station 1 had the greatest ADG and the greatest increase in ADG, whereas station 6, which also had clear improvements in blood measures, had the lowest ADG of all stations and was not responsive for ADG. The greatest numerical BW at the time of administration of the second injection (which would to some degree be a function of the first injection), along with the greatest numerical response in Hb concentration and increase in ADG to the second injection, all appeared at the station where pigs were receiving the intermediate dose of iron. The total amount of iron injected at station 6 from the 2 doses would have equaled the amount injected in just a single dose at station 3. Although some of the differences in response seen between those 2 stations could be postulated to be a function of total iron supply, the difference that then is seen with station 1, where pigs received the intermediate total supply of iron, clouds the picture. Clearly, whereas iron status may be limiting to growth in some situations, it is not the singular controlling factor in all situations, requiring further studies.

Tissue Mineral Content

Liver, spleen, heart, and kidney samples were analyzed for mineral content from barrows at 1 of the 7 research stations at d −4, 0, 14, and 28 (Table 11) relative to weaning. The use of only 4 pigs per treatment for the tissue mineral content was low statistical power that limited somewhat the interpretation of the results. Nevertheless, patterns of change in mineral content, particularly Fe, were evident. In the CON pigs, the liver iron content decreased at weaning relative to the d −4 sample, being only 55% at weaning. This is unsurprising given that the pigs were growing

Table 11. Effects of an additional iron injection on liver, spleen, heart, and kidney mineral concentration from pigs at station 1 (DM basis, mg/kg)[1]

	Liver			Spleen			Heart			Kidney		
Item	CON	+Fe	SEM	CON	+Fe	SEM	CON	+Fe	SEM	CON	+Fe	SEM
d −4												
Fe	495.1			151.1			234.6			179.8		
Zn	305.3			60.2			61.4			73.1		
Cu	373.3			4.3			16.5			33.5		
d 0												
Fe	274.4	809.2	125.88[2]	750.6	1,024.2	100.01[3]	170.1	270.4	37.62	138.8	252.9	45.19
Zn	346.0	316.2	63.11	88.5	90.1	8.9	63.7	79.9	10.57	77.3	79.2	1.38
Cu	392.3	393.9	53.94	6.7	4.8	0.93	15.4	19.1	2.88	35.9	38.6	3.56
d 14												
Fe	476.5	536.4	36.26	802.6	885	82.92	251.2	266.8	30.72	247.9	396.7	71.57
Zn	300.4	309.0	23.64	159.0	150.5	8.39	62.4	58.8	2.08	81.0	82.1	2.52
Cu	103.4	118.5	28.88	3.6	3.6	1.04	16.0	13.8	0.53[4]	32.8	34.3	2.77
d 28												
Fe	598.9	600.4	41.29	711.1	847.1	117.15	202.4	240.2	23.15	368.5	367.3	29.1
Zn	427.8	412.1	56.53	160.2	152.1	11.52	63.5	59.8	1.52	94.5	82.8	5.73
Cu	19.1	8.3	3.99	2.3	2.1	0.09	16.0	15.3	1.09	41.5	32.3	3.73

[1]All pigs received an initial iron injection at processing of the litter following birth. The +Fe group received an additional iron injection 3 to 5 d before weaning (d 0). Means at d −4 represent 8 barrows; treatment means at d 0, 14, and 28 represent 4 barrows per treatment. CON = control group; +Fe = the added-injection group. The average DM contents for liver, spleen, heart, and kidney were 23.3 ± 0.5%, 20.0 ± 0.3%, 20.3 ± 0.8%, and 16.9 ± 0.5%, respectively, throughout the experiment.

[2]Treatment effect (P = 0.02).

[3]Tendency for a treatment effect (P = 0.10).

[4]Treatment effect (P = 0.03).

and sow milk contains very little Fe. However, the liver iron content of the +Fe pigs increased to 163% of the d −4 value in response to the injection and clearly differed ($P = 0.02$) at weaning. However, by d 14 after weaning the significant treatment difference that existed at weaning was absent for the +Fe group, and liver content became essentially identical by d 28 after weaning. Liver Zn and Cu content were not affected for the +Fe group as both the CON and +Fe groups were similar at all periods of the experiment. However, there was a drastic decline in liver Cu for both treatments from weaning to d 28 after weaning.

Iron content of the spleen (Table 11) tended to be greater ($P = 0.10$) for the +Fe pigs compared with the CON pigs at weaning but not at d 14 and 28 of the nursery. The spleen iron content was 5 to 6 times greater at weaning than at d −4 and maintained that increase for the rest of the study. Similar to liver iron content, the heart and kidney iron content in the CON pigs was numerically less at weaning compared with d −4 but greater for the +Fe pigs. Although the heart and kidney Fe content followed the pattern of the liver and spleen, and was 159% and 182% for the +Fe pigs compared with the CON pigs, respectively, the values were not significant ($P = 0.12$ and 0.11, respectively). Additionally, the heart and kidney Cu concentration remained relatively constant from d −4 to the end of the experiment (d 28 after weaning) compared with the liver and spleen, where there was at least a 50% decrease in Cu concentration from weaning to d 28. The increases in iron content of the tissues from the +Fe pigs at weaning are obviously due to the additional iron provided in the second injection. It has been proposed (Danielson, 2004) that an i.m. injection of iron dextran is absorbed by the body relatively fast through the reticuloendothelial system because of the phagocytes in the liver, spleen, and bone marrow. Thus, this could explain the large increase in iron content observed in only a 4-d period. Furthermore, Pu et al. (2018) reported observations of iron accumulation in the liver of young pigs, as well as serum iron, ferritin, and transferrin as early as d 5 of age when iron injections were administered on d 3 of life.

The liver, heart, and kidneys from pigs not receiving a second iron injection all had decreased iron concentrations from d 4 to weaning. The reduction in tissue iron concentration is presumably from the body mobilizing iron from multiple tissue reserves to support normal erythropoiesis and hemoglobin synthesis. Furthermore, a normal blood hemoglobin concentration, but reduced liver iron concentration, can indicate the beginning of iron deficiency as hemoglobin synthesis will mobilize iron from body reserves

(Dallman, 1986; Conrad and Umbreit, 2002). In the current experiment, it is suggestive that the CON pigs may be in an iron-deficient state as liver, kidney, and heart iron content is decreasing from d −4 to 0 (weaning).

APPLICATIONS

In summary, it is well understood that even after an initial iron injection at postfarrowing litter processing, a large proportion of piglets reared from modern sows become iron deficient before weaning. Thus, it was warranted to evaluate the effects of an additional iron injection before weaning. Providing an additional iron injection before weaning could theoretically benefit fast-growing piglets and help bridge the potential iron gap around weaning. The station with the heaviest or fastest growing pigs in the present study also happened to have the greatest magnitude of response from the additional iron injection. However, there were instances where hematological responses occurred with no growth response; thus, improved hematological status by a second iron injection close to weaning with the same dose of iron that is administered to piglets at birth does not always translate to improved ADG. Therefore, it is suggested that iron status was not the limiting factor for growth performance when a sufficient amount of dietary iron is provided to piglets after weaning close to 21 d of age for several of the stations that lacked a response in ADG. Overall, it is likely that the benefits of an additional iron injection before weaning is dependent on individual herd characteristics and health status; the identification of the differences between individual production settings that are associated with responsiveness to an additional iron injection will require further research.

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LITERATURE CITED

Bhattarai, S., and J. P. Nielsen. 2015a. Early indicators of iron deficiency in large piglets at weaning. J. Swine Health Prod. 23:10–17.

Bhattarai, S., and J. P. Nielsen. 2015b. Association between hematological status at weaning and weight gain post-weaning in piglets. Livest. Sci. 182:64–68. https://doi.org/10.1016/j.livsci.2015.10.017.

Chevalier, T. B., W. Lyons, D. B. Paczosa, G. K. Rentfrow, and M. D. Lindemann. 2023. A second iron injection administered to piglets during lactation improves hemoglobin concentration, growth performance, and carcass characteristics at slaughter. J. Anim. Sci. 101:1–8. https://doi.org/10.1093/jas/skad270.

Chevalier, T. B., H. J. Monegue, and M. D. Lindemann. 2021. Effects of iron dosage administered to newborn piglets on hematological measures, preweaning and postweaning growth performance, and postweaning tissue mineral content. J. Swine Health Prod. 29:189–199. https://doi.org/10.54846/jshap/1207.

Conrad, M. E., and J. N. Umbreit. 2002. Pathways of iron absorption. Blood Cells Mol. Dis. 29:336–355. https://doi.org/10.1006/bcmd.2002.0564.

Dallman, P. 1986. Biochemical basis for the manifestations of iron deficiency. Annu. Rev. Nutr. 6:13–40. https://doi.org/10.1146/annurev.nu.06.070186.000305.

Danielson, B. G. 2004. Structure, chemistry, and pharmacokinetics of intravenous iron agents. J. Am. Soc. Nephrol. 15:S93–98. https://doi.org/10.1097/01.ASN.0000143814.49713.C5.

Egeli, A. K., and T. Framstad. 1998. Evaluation of the efficacy of perorally administered glutamic acid-chelated iron and iron-dextran injected subcutaneously in Duroc and Norwegian Landrace piglets. J. Vet. Med. A Physiol. Pathol. Clin. Med. 45:53–61. https://doi.org/10.1111/j.1439-0442.1998.tb00800.x.

FASS. 2020. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 4th ed. FASS Inc.

Fredericks, L., C. Olsen, A. Maschhoff, and C. Shull. 2018. Evaluation of the impact of iron dosage on post-weaning weight gain, and mortality. Page 315 in Proc. Am. Assoc. Swine Vet., San Diego, CA. American Association of Swine Veterinarians.

Haugegaard, J., H. Wachmann, and P. Kristensen. 2008. Effect of supplementing fast-growing, late-weaned piglets twice with 200 mg iron dextran intra-muscularly. Pig J. 61:69–73.

Holter, P. H., T. Framstad, A. Aulie, H. E. Refsum, and Ø. V. Sjaastad. 1991. Effect of iron treatment on erythrocyte parameters in postnatal anemia of the pig. Pediatr. Hematol. Oncol. 8:1–11. https://doi.org/10.3109/08880019109033422.

Jolliff, J. S., and D. C. Mahan. 2011. Effect of injected and dietary iron in young pigs on blood hematology and postnatal pig growth performance. J. Anim. Sci. 89:4068–4080. https://doi.org/10.2527/jas.2010-3736.

Kamphues, J., K. Manner, and C. Netzer. 1992. Effects of a 2nd iron injection in suckling piglets on iron retention and performance before and after weaning. Page 601 in Proc. 12th Int. Pig Vet. Congr., the Hague, the Netherlands. International Pig Veterinary Society.

Merck. 2016. The Merck Veterinary Manual. 11th ed. Merck & Co. Inc., Kenilworth NJ.

NASEM (National Academies of Sciences, Engineering, and Medicine). 2012. Nutrient Requirements of Swine. 11th rev. ed. National Academies Press, Washington, DC.

Olsen, C. 2020. Field trial results: A second dose of injectable iron to maximize growth. Pages 157–158 in Proc. Am. Assoc. Swine Vet., Atlanta, GA. American Association of Swine Veterinarians.

Perri, A. M., R. M. Friendship, J. C. S. Harding, and T. L. O’Sullivan. 2016. An investigation of iron deficiency and anemia in piglets and the effect of iron status at weaning on post-weaning performance. J. Swine Health Prod. 24:10–20.

Pu, Y., S. Li, H. Xiong, X. Zhang, Y. Wang, and H. Du. 2018. Iron promotes intestinal development in neonatal piglets. Nutrients 10:726. https://doi.org/10.3390/nu10060726.

Thorn, C. E. 2010. Hematology of the pig. Pages 843–851 in Shalm’s Veterinary Hematology. 6th ed. D. J. Weiss and J. K. Wardrop, ed. Wiley-Blackwell., Ames, IA.

Van Gorp, S., H. Segers, and C. von der Recke. 2012. Preventing iron deficiency by avoiding an iron gap in modern pig production. Pages 407–408 in Proc. Am. Assoc. Swine Vet., Denver, CO. American Association of Swine Veterinarians.

Venn, J., R. McCance, and E. Widdowson. 1947. Iron metabolism in piglet anaemia. J. Comp. Pathol. Ther. 57:314–325. https://doi.org/10.1016/S0368-1742(47)80037-2.

Williams, H. E., J. M. DeRouchey, J. C. Woodworth, S. S. Dritz, M. D. Tokach, R. D. Goodband, A. J. Holtcamp, E. M. Bortoluzzi, and J. T. Gebhardt. 2020. Effects of increasing Fe dosage in newborn pigs on suckling and subsequent nursery performance and hematological and immunological criteria. J. Anim. Sci. 98:skaa221. https://doi.org/10.1093/jas/skaa221.

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