Effect of iron injection strategies for suckling piglets on pre- and post-weaning growth performance and hematocrit levels

Efecto de varias estrategias de inyección de hierro en lechones lactantes sobre el rendimiento del crecimiento antes y después del destete y los niveles de hematocrito

Efeito das estratégias de injeção de ferro em leitões no desempenho de crescimento pré e pós-desmame e nos níveis de hematócrito

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Abstract

Background: A single, 200-mg iron injection for newborn pigs is a common practice in swine production. It is required to provide sufficient iron for newborn pigs while avoiding the potential risk of excessive amount of iron causing oxidative stress. **Objective:** To evaluate the quantity and timing of a single or second iron injection into suckling piglets on growth performance and hematocrit levels. **Methods:** A total of 24 piglets from four litters were used in each experiment (3). Treatments were: Experiment 1- 1) Control: 150 mg iron injection at d 1-3 of age, 2) Iron14: additional 100 mg iron injection at 14 d before weaning, and 3) Iron7: additional 100 mg iron injection at 7 d before weaning; Experiment 2- 1) Control: 100 mg iron injection at d 1-3 of age, 2) Iron100/100: 100 mg iron at d 1-3 of age and 9 d after the first injection, and 3) Iron200: 200 mg iron injection at d 1-3 of age; and Experiment 3 (at d 3-4 of age)- 1) Fe100: 100 mg iron injection, 2) Fe200: 200 mg iron injection, and 3) BW200: 125 mg iron injection/kg body weight. Pigs were weaned at d 25-28 of age. Growth performance and hematocrit levels were measured until d 13-14 postweaning. **Results:** In Experiment 1, the Iron14 treatment had a greater final body weight than the control treatment (p=0.07). Hematocrit levels were greater in the Iron14 (p=0.10) and Iron7 (p<0.05) treatments than in the control treatment at d 14 postweaning. In Experiment 2, average daily gain in the postweaning period was greater in the Iron100/100 treatment than in the control (p<0.05) and Iron200 (p=0.08) treatments. Hematocrit levels in the Iron200 treatment were greater than those in the control treatment (p<0.05) at d 13 postweaning. In Experiment 3, there was no difference in growth performance, but the BW200 treatment increased hematocrit levels to a greater degree than the Fe200 treatment (p<0.10). **Conclusion:** An additional iron injection earlier and a split iron injection to newborn pigs could benefit postweaning growth, but not by body weight-based iron injection. Hematocrit levels increase with a greater dose of iron injected although the magnitude varies depending on injection strategies.

Keywords: *anaemia; body weight; growth; hematocrit; injection; iron; newborn; oxidative stress; pig; postweaning.*

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Resumen

Antecedentes: Una única inyección de hierro de 200 mg para cerdos recién nacidos es una práctica común en la producción porcina. Es necesario proporcionar suficiente hierro a los cerdos recién nacidos y, al mismo tiempo, evitar el riesgo potencial de que una cantidad excesiva de hierro cause estrés oxidativo. **Objetivo:** Evaluar la cantidad y el momento de una única o segunda inyección de hierro en lechones lactantes sobre el rendimiento del crecimiento y los niveles de hematocrito. **Métodos:** En cada experimento (3) se utilizaron un total de 24 lechones de 4 camadas. Los tratamientos fueron: *Experimento 1*: 1) Control: inyección de 150 mg de hierro al día 1-3 de edad, 2) Hierro14: inyección adicional de 100 mg de hierro a los 14 días antes del destete, y 3) Hierro7: inyección adicional de 100 mg de hierro a los 7 días antes del destete; *Experimento 2*: 1) Control: inyección de 100 mg de hierro al día 1-3 de edad, 2) Hierro100/100: 100 mg de hierro al día 1-3 de edad y 9 días después de la primera inyección, y 3) Hierro200: 200 mg inyección de hierro al día 1-3 de edad; y *Experimento 3* (a los 3-4 días de edad): 1) Fe100: inyección de 100 mg de hierro, 2) Fe200: inyección de 200 mg de hierro y 3) BW200: inyección de 125 mg de hierro/kg de peso corporal. Los cerdos fueron destetados entre los 25 y 28 días de edad. El rendimiento del crecimiento y los niveles de hematocrito se midieron hasta el día 13-14 después del destete. **Resultados:** En el *Experimento 1*, el tratamiento con Hierro14 tuvo un mayor peso corporal final que el tratamiento control (p=0,07). Los niveles de hematocrito fueron mayores en los tratamientos Hierro14 (p=0,10) y Hierro7 (p<0,05) que en el tratamiento control el día 14 post-destete. En el *Experimento 2*, la ganancia diaria promedio en el período posdestete fue mayor en el tratamiento Hierro100/100 que en los tratamientos control (p<0,05) y Hierro200 (p=0,08). Los niveles de hematocrito en el tratamiento con Hierro200 fueron mayores que los del tratamiento control (p<0,05) en el día 13 después del destete. En el *Experimento 3*, no hubo diferencias en el rendimiento del crecimiento, pero el tratamiento con BW200 aumentó los niveles de hematocrito en mayor grado que el tratamiento con Fe200 (p<0,10). **Conclusión:** Una inyección adicional de hierro más temprana y una inyección dividida a los cerdos recién nacidos podrían beneficiar el crecimiento post-destete, pero no basada en el peso corporal. Los niveles de hematocrito aumentan con una mayor dosis de hierro inyectada, aunque la magnitud varía según la estrategia de inyección.

Palabras clave: *anemia; cerdo recién nacido; crecimiento; estres oxidativo; hematocrito; hierro; inyección; lechón; peso corporal; post-destete.*

Resumo

Antecedentes: A suplementação injetável de ferro ao nascimento é uma prática para garantir a saúde e o desenvolvimento adequado de leitões neonatos. Uma deficiência pode predispor os animais à anemia, enquanto uma superdose pode induzir estresse oxidativo. **Objetivo:** Avaliar a quantidade e o momento de uma ou duas injeções de ferro em leitões lactentes na performance de crescimento e nos níveis de hematócrito. **Métodos:** Um total de 24 leitões de 4 leitegadas foram usados em cada experimento (3). Os tratamentos foram: Experimento 1- 1) Control: injeção de 150 mg de ferro nos dias 1-3 de idade, 2) Iron14: injeção adicional de 100 mg de ferro 14 dias antes do desmame, e 3) Iron7: injeção adicional de 100 mg de ferro 7 dias antes do desmame; Experimento 2- 1) Control: injeção de 100 mg de ferro nos dias 1-3 de idade, 2) Iron100/100: 100 mg de ferro nos dias 1-3 de idade e 9 dias após a primeira injeção, e 3) Iron200: injeção de 200 mg de ferro nos dias 1-3 de idade; e Experimento 3 (nos dias 3-4 de idade)- 1) Fe100: Injeção de 100 mg de ferro, 2) Fe200: Injeção de 200 mg de ferro, e 3) BW200: Injeção de 125 mg de ferro por kg de peso corporal. Os leitões foram desmamados nos dias 25-28 de idade. O desempenho de crescimento e os níveis de hematócrito foram medidos até os dias 13-14 pós-desmame. **Resultados:** No Experimento 1, Iron14 teve maior peso corporal final comparado ao Control (p=0,07). Os níveis de hematócrito foram maiores nos tratamentos Iron14 (p=0,10) e Iron7 (p<0,05) do que no Control no dia 14 após o desmame. No Experimento 2, o ganho médio diário no período pós-desmame foi maior no Iron100/100 do que no Control (p<0,05) e no Iron200 (p=0,08). Os níveis de hematócrito no Iron200 foram maiores do que no Control (p<0,05) no dia 13 após o desmame. No Experimento 3, não houve diferença no desempenho de crescimento, mas o tratamento BW200 aumentou os níveis de hematócrito em maior grau do que o Fe200 (p<0,10). **Conclusão:** Uma injeção adicional de ferro mais cedo e a divisão da injeção de ferro para leitões recém-nascidos podem beneficiar o crescimento pós-desmame, mas não com base no peso corporal para a suplementação injetável de ferro. Os níveis de hematócrito aumentaram com uma maior dose de ferro injetado, embora a magnitude varie dependendo das estratégias de suplementação.

Palavras-chave: *anemia; crescimento; estresse oxidativo; ferro; hematócrito; injeção; leitão; peso corporal; porco; pós-desmame; recém-nascido.*

Introduction

Iron plays a crucial role in various metabolic processes, such as oxygen transport, DNA synthesis, energy metabolism, and neuronal development (Dong *et al.*, 2020). Newborn piglets face a significant risk of iron deficiency due to limited iron reserves and low iron content in sow milk, and iron deficiency often leads to anemia (Perri *et al.*, 2016; Szudzik *et al.*, 2018). Therefore, it is common practice to administer a 200 mg iron within the first week of a piglet's life (Williams *et al.*, 2021) as it enhances growth performance, hemoglobin levels, and iron status in piglets' bodies (Chevalier *et al.*, 2021). However, with the extension of the weaning age varying from 18 to 28 days, hemoglobin and hematocrit levels could decline from day 21 of age until weaning, potentially resulting in iron deficiency anemia in weaning pigs due to low iron intake in the first week after weaning (Lipiński *et al.*, 2010; Joliff and Mahan, 2011; Perri *et al.*, 2016). However, administering an excess of iron to pigs at birth could be harmful; it could trigger hydroxyl radical formation and damage biological molecules in the body (Szudzik *et al.*, 2018). To address these challenges, the strategy of a split and a body weight-based iron injection have been proposed to maintain iron injection efficacy while minimizing potential toxicity to newborn piglets. In addition, it has been reported that an additional dose of iron before weaning could improve hemoglobin and hematocrit levels and postweaning performance (Joliff and Mahan, 2011; Albers *et al.*, 2022; Chevalier *et al.*, 2023). However, these studies lack information regarding when a second iron injection is needed to maximize its efficiency in improving growth performance and hematological status, as Albers *et al.* (2022) reported no difference in preweaning growth performance by a second iron injection before weaning, and injected iron takes time to be involved in iron metabolism and eventually utilized in hemoglobin synthesis (Chevalier *et al.*, 2021). Therefore, three independent studies were conducted with the primary aim of evaluating the quantity, timing, and effects of a single and a second iron injection into suckling piglets, focusing on its impact on pre- and post-weaning growth performance and hematocrit levels.

Materials and Methods

Ethical considerations

All procedures used in the three experiments were approved by the Institutional Animal Care and Use Committee of the University of Wisconsin-River Falls (Protocol # 20-21-33030; 20-21-33041; 20-21-33045 for Experiment 1, 2, and 3, respectively).

Location

All three experiments were conducted in the nursery facility at Mann Valley Farm of the University of Wisconsin-River Falls (WI, USA).

Animals, experimental design, and treatment

To evaluate the effect of an additional iron injection at various ages before weaning in Experiment 1, a total of 24 suckling piglets (Yorkshire × Yorkshire, Yorkshire × Duroc, and Yorkshire × Duroc × Duroc; initial body weight, 1.62±0.12 kg) from four litters at d 1 to 3 of age (d 0 of experiment) were allotted into 3 treatments within litter (8 pigs per treatment; 4 barrows and 4 gilts) based on body weight and gender, as follows: 1) Control: 150 mg intramuscular iron-dextran at d 1 to 3 of age (d 0 of experiment), 2) Iron14: additional intramuscular injection of 100 mg iron-dextran at 14 d prior to weaning, and 3) Iron7: additional intramuscular injection of 100 mg iron-dextran at 7 d prior to weaning. The initial 150 mg iron-dextran was injected into all piglets intramuscularly on the initial day (d 1 to 3 of age) with an additional injection given to the piglets either 7 or 14 d before weaning, except for the control treatment. A commercial iron-dextran product (Durvet, Inc., Springs, MO, USA) was used for the iron injections for all piglets in all three experiments. Weaning age was at d 25-28 of age (d 23-24 of the experiment).

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To evaluate the effect of split iron injection in Experiment 2, a total of 24 suckling piglets (Yorkshire × Yorkshire, Yorkshire × Duroc, and Yorkshire × Duroc × Duroc; initial body weight, 2.19±0.15 kg) from four litters at d 1 to 3 of age (d 0 of experiment) were allotted into 3 treatments within litter (8 pigs per treatment; 4 barrows and 4 gilts) based on body weight and gender, as follows: 1) Control: 100 mg iron at d 0 of experiment + no additional iron injection, 2) Iron100/100: 100 mg iron at d 0 of experiment + 100 mg iron injection at d 9 of experiment (d 14 before weaning), and 3) Iron200: 200 mg iron at d 0 of experiment + no additional Fe injection. The 100 or 200 mg of iron-dextran were injected into all piglets intramuscularly on the initial day (d 1 to 3 of age) with the following injection given to the piglets 14 d before weaning only for the Iron100/100 treatment.

To evaluate the effect of iron injection based on piglet body weight in Experiment 3, a total of 24 piglets (Yorkshire × Yorkshire, Yorkshire × Duroc, and Yorkshire × Duroc × Duroc; initial body weight: 1.55±0.11 kg) from four litters at d 3 to 4 of age (d 0 of experiment), were allotted into 3 treatments within litter (8 pigs per treatment; 4 barrows and 4 gilts) based on body weight and gender, as follows: 1) Fe100: 100 mg iron at d 0 of experiment, 2) Fe200: 200 mg iron at d 0 of experiment, and 3) BW200: 125 mg iron per kg body weight at d 0 of experiment targeting 200 mg of iron injection for pigs weighing an average 1.6 kg based on initial body weight in this experiment. Iron-dextran were injected into all piglets intramuscularly on the initial day (d 3 to 4 of age).

Housing and diet

For all 3 experiments, all sows with suckling piglets across the three experiments were housed in raised-deck farrowing crates (1.52 × 2.13 m^2) with tenderfoot or woven-wire flooring in an environmentally controlled farrowing facility. A typical corn-soybean meal-based lactation diet was provided to all sows *ad libitum* with free access to water from a water nipple throughout the entire lactation period. All piglets were processed (weighing, ear-notching, tail docking, and castration) at the time of the first iron injection. No creep feed was offered to piglets throughout the suckling period. All piglets were weaned at d 23-24 of the experiment (d 25-28 of age).

At weaning, pigs in each experiment were weaned and housed by litter in raised-deck nursery pens (1.32 × 1.63 m^2) with plastic or woven-wire flooring in an environmentally controlled nursery facility for a 2-week growth period. All pigs were fed *ad libitum* a common nursery diet and given free access to water for a 14-d nursery period. The nursery diet was a corn-soybean meal-based diet with 15% whey, 2.5% blood meal, and 2.25% fish meal that contained 3,250 kcal metabolizable energy/kg, 1.42% standardized ileal digestible lysine, and 277 mg iron/kg.

Data and sample collection

In Experiment 1, pigs were weighed, and blood samples were collected from all piglets at d 0 (initial), 10 (additional iron injection for the Iron14 treatment), 17 (additional iron injection for the Iron7 treatment), 24 (weaning), 31, and 38 of experiment. In Experiment 2, pigs were weighed, and blood samples were collected from all piglets at d 0 (initial), 9 (second dose for the Iron100/100 treatment), 16, 23 (weaning), 30, and 36 of the experiment. In Experiment 3, pigs were weighed, and blood samples were collected from all piglets at d 0 (initial), 10, 17, 24 (weaning), 31, and 38 of the experiment.

In the nursery period, body weight and feed consumption were recorded at d 7 and 14 postweaning and average daily gain (ADG) was calculated in all three experiments. Blood sampling procedure was the same across all three experiments. Blood samples were collected from the jugular vein into a glass tube containing K_3 EDTA (Greiner Bio-One, Monroe, NC, USA) on the same days of body weight measurement for determination of hematocrit levels. For hematocrit level determination, two 75 mm sodium-heparinized capillary tubes (Jorgensen Laboratories Inc., Loveland, Colorado USA) were filled with blood from each blood sample and then packed with clay prior to being put in the microhematocrit centrifuge (UNICO, Dayton, New Jersey, USA). After the samples were spun down at 10,000 *g* for 6 minutes at room temperature and the plasma and red blood cells were separated, the capillary tubes were placed on a microhematocrit reader (Jorgensen Laboratories Inc., Loveland, Colorado USA), and the hematocrit level was determined in duplicate by two trained observers.

Statistical analysis

Growth performance and blood data from the three experiments were analyzed individually with ANOVA for a randomized complete block design using PROC MIXED of SAS (version 9.2; SAS Inst. Inc., Cary, NC, USA). Single degree of freedom contrasts were conducted to compare the three treatments. The experimental unit was the individual pig within litter or pen. Models included the treatment as a fixed effect and the replicate within the litter (or pen), litter (or pen), and litter (or pen) × treatment as random effects. Outlier analysis within each treatment and day was performed using the Grubb's test outlier calculator (GraphPad Software, San Diego, CA) and no outlier was identified. Least square means were separated using the PDIFF option of SAS. Statistical differences were considered significant at p<0.05 and tendency at p<0.10.

Results

In Experiment 1, the Iron14 treatment had a greater final body weight at d 38 of experiment compared with the control treatment (p=0.07, tendency) (Table 1). In the overall 14-d postweaning period, the Iron14 treatment had numerically greater ADG than the control and Iron7 treatments (p=0.12).

Hematocrit levels in Experiment 1 (Table 2) were numerically greater in the Iron7 treatment than the control treatment at d 10 of experiment (p=0.11). Hematocrit levels were greater in the Iron14 treatment than the control treatment at d 17, 24, 31 (p<0.05), and 38 (p=0.10, tendency) of experiment whereas those were greater in the Iron7 treatment than the control treatment at d 24, 31, and 38 of experiment (p<0.05). At d 17 of experiment,

Table 1. Growth performance in pre- and postweaning periods in Experiment 1.

	Treatment[1]				P-value[2]		
	Control	Iron14	Iron7	SEM	1 vs 2	1 vs 3	2 vs 3
Body weight, kg							
0 d of exp.	1.60	1.61	1.63	0.12	0.92	0.61	0.68
10 d of exp.[3]	3.86	3.96	3.93	0.47	0.69	0.77	0.92
17 d of exp.[4]	5.63	5.39	5.76	0.64	0.53	0.75	0.35
24 d of exp.(wean)[5]	6.85	7.30	7.64	0.74	0.50	0.27	0.60
31 d of exp.[6]	7.36	8.26	7.94	0.71	0.19	0.39	0.62
38 d of exp.[7]	9.38	10.97	10.15	0.99	0.07	0.34	0.30
Average daily gain, g/d							
0-24 d of exp.	218.08	236.74	249.61	28.55	0.49	0.27	0.63
24-38 d of exp.	190.31	263.80	189.72	28.87	0.12	0.99	0.12

[1] 1) Control: No secondary iron injection with 150 mg of iron injection at d 1 to 3 of age, 2) Iron14: Secondary intramuscular injection of 100 mg iron-dextran14 d before weaning (d 10 of experiment), and 3) Iron7: Secondary intramuscular injection of 100 mg iron-dextran 7 d before weaning (d 17 of experiment).
[2] n=8 per treatment.
[3] Second iron injection for the Iron14 treatment.
[4] Second iron injection for the Iron7 treatment.
[5] Weaning day.
[6] d 7 postweaning.
[7] d 14 postweaning.

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the Iron14 treatment had greater hematocrit levels than the Iron7 treatment ($p<0.05$).

In Experiment 2, the Iron100/100 treatment had greater body weight than the control treatment ($p<0.05$) at d 30 and 36 of experiment (Table 3), whereas the Iron200 treatment tended to have greater body weight ($p=0.06$) than the control treatment at d 30 of experiment.

Table 2. Hematocrit levels (%) in pre- and postweaning periods in Experiment 1.

	Treatment[1]				P-value[2]		
	Control	Iron14	Iron7	SEM	1 vs 2	1 vs 3	2 vs 3
Hematocrit, %							
0 d of exp.	25.47	24.88	23.87	2.10	0.61	0.21	0.41
10 d of exp.[3]	35.35	36.75	36.92	1.07	0.14	0.11	0.84
17 d of exp.[4]	36.01	41.94	35.94	1.57	0.02	0.97	0.02
24 d of exp.(wean)[5]	32.55	37.81	35.91	1.23	0.01	0.04	0.19
31 d of exp.[6]	32.82	38.38	38.11	1.15	0.01	0.01	0.85
38 d of exp.[7]	29.54	32.57	33.59	1.24	0.10	0.04	0.53

[1] 1) Control: No secondary iron injection with 150 mg of iron injection at d 1 to 3 of age, 2) Iron14: Secondary intramuscular injection of 100 mg iron-dextran14 d before weaning (d 10 of experiment) and 3) Iron7: Secondary intramuscular injection of 100 mg iron-dextran 7 d before weaning (d 17 of experiment).
[2] n=8 per treatment.
[3] Second iron injection for the Iron14 treatment.
[4] Second iron injection for the Iron7 treatment.
[5] Weaning day.
[6] d 7 postweaning.
[7] d 14 postweaning.

Table 3. Growth performance in pre- and postweaning periods in Experiment 2.

	Treatment[1]				P-value[2]		
	Control	Iron100/100	Iron200	SEM	1 vs 2	1 vs 3	2 vs 3
Body weight, kg							
0 d of exp.	2.19	2.20	2.19	0.15	0.92	0.99	0.91
9 d of exp.[3]	4.52	4.75	4.56	0.38	0.36	0.86	0.46
16 d of exp.	6.59	7.05	6.85	0.57	0.35	0.59	0.68
23 d of exp.(wean)[4]	8.68	9.45	9.27	0.71	0.27	0.40	0.78
30 d of exp.[5]	8.35	9.79	9.59	0.65	0.03	0.06	0.72
36 d of exp.[6]	10.17	12.55	11.45	0.88	0.01	0.12	0.16
Average daily gain, g/d							
0-23 d of exp.	284.04	315.46	307.44	26.21	0.30	0.45	0.78
23-36 d of exp.	111.36	238.20	156.31	35.37	0.01	0.31	0.08

[1] 1) Control: 100 mg Fe at d 0 of experiment (d 1 to 3 of age) + no additional Fe injection, 2) Iron100/100: 100 mg Fe at d 0 of experiment + 100 mg Fe injection at d 9 of experiment (d 14 before weaning), and 3) Iron200: 200 mg Fe at d 0 of experiment + no additional Fe injection.
[2] n=8 per treatment.
[3] Second iron injection for the iron100/100 treatment.
[4] Weaning day.
[5] d 7 postweaning.
[6] d 13 postweaning.

Postweaning ADG was greater in the Iron100/100 treatment than in the control ($p<0.05$) and Iron200 ($p=0.08$, tendency) treatments.

Hematocrit levels in Experiment 2 (Table 4) were greater in the Iron100/100 treatment than the control treatment at d 16, 23, 30 ($p<0.05$) and 36 ($p=0.10$, tendency) of experiment. The Iron200 treatment had greater hematocrit levels than the control treatment at d 9 to 36 of experiment ($p<0.05$). The Iron100/100 treatment had a lower hematocrit level at d 9 ($p<0.05$) and 36 ($p=0.10$, tendency) of experiment than the Iron200 treatment.

In Experiment 3, there was no difference in pre- and postweaning growth performance (Table 5).

Table 4. Hematocrit levels (%) in pre- and postweaning periods in Experiment 2.

	Treatment[1]			SEM	P-value[2]		
	Control	Iron100/100	Iron200		1 vs 2	1 vs 3	2 vs 3
Hematocrit, %							
0 d of exp.	21.89	20.81	21.75	1.36	0.43	0.92	0.49
9 d of exp.[3]	30.35	29.88	33.85	1.34	0.73	0.04	0.02
16 d of exp.	27.69	34.38	34.67	1.81	0.01	0.01	0.87
23 d of exp.(wean)[4]	25.61	32.13	31.42	1.60	0.01	0.01	0.68
30 d of exp.[5]	32.06	37.06	38.10	1.31	0.01	0.01	0.44
36 d of exp.[6]	29.73	31.56	33.54	0.84	0.10	0.01	0.10

[1] 1) Control: 100 mg Fe at d 0 of experiment (d 1 to 3 of age) + no additional Fe injection, 2) Iron100/100: 100 mg Fe at d 0 of experiment + 100 mg Fe injection at d 9 of experiment (d 14 before weaning), and 3) Iron200: 200 mg Fe at d 0 of experiment + no additional Fe injection.
[2] n=8 per treatment.
[3] Second iron injection for the iron100/100 treatment.
[4] Weaning day.
[5] d 7 postweaning.
[6] d 13 postweaning.

Table 5. Growth performance in pre- and postweaning periods in Experiment 3.

	Treatment[1]			SEM	P-value[2]		
	Control	Fe200	BW200		1 vs 2	1 vs 3	2 vs 3
Body weight, kg							
0 d of exp.	1.55	1.54	1.54	0.11	0.94	0.91	0.96
10 d of exp.	3.82	3.82	3.69	0.22	1.00	0.51	0.49
17 d of exp.	5.71	5.97	5.55	0.34	0.43	0.63	0.21
24 d of exp.(wean)[3]	7.38	7.36	7.34	0.58	0.98	0.95	0.98
31 d of exp.[4]	8.11	8.64	8.26	0.55	0.44	0.83	0.55
38 d of exp.[5]	10.91	11.53	10.90	0.63	0.47	0.99	0.45
Average daily gain, g/d							
0-24 d of exp.	243.21	242.29	241.48	21.49	0.97	0.94	0.97
24-38 d of exp.	250.94	274.62	253.43	26.80	0.49	0.94	0.52

[1] 1) Fe100: 100 mg iron at d 0 of experiment (d 3 to 4 of age), 2) Fe200: 200 mg Fe at d 0 of experiment, and 3) BW200: 125 mg Fe per kg body weight at d 0 of experiment targeting 200 mg of iron injection for pigs weighing an average of 1.6 kg.
[2] n=8 per treatment.
[3] Weaning day.
[4] d 7 postweaning.
[5] d 14 postweaning.

Hematocrit levels in Experiment 3 (Table 6) were higher in the Fe200 treatment than the Fe100 treatment at d 10 and 31 of experiment (p<0.05). The BW200 treatment had greater hematocrit levels than the control treatment at d 10 to 24 (p<0.10, tendency) and 31 (p<0.05) of experiment. There was no difference in hematocrit levels between the Fe200 and BW200 treatments.

Discussion

In swine production, a 200 mg intramuscular iron injection is a common practice to prevent piglets from iron deficiency anemia as piglets have low iron reserves in the body at birth, and sow milk only contains 0.2-4 mg/L of iron, which is not sufficient to meet the daily iron requirement of suckling piglets as piglets may only absorb approximately 1 mg of iron per day while requiring about 7 mg of iron per day (NRC, 2012; Szudzik *et al.*, 2018). Newborn piglets receiving an iron injection below 100 mg at d 1 of age showed hemoglobin levels below 9 g/dL at weaning, which is considered anemic (Chevalier *et al.*, 2023). Therefore, it is critical to provide enough iron to pigs to meet their iron needs during the suckling and nursery periods to improve their growth and hematological status (Albers *et al.*, 2022). In the current studies, growth performance in pre- and post-weaning periods and hematocrit levels were measured for all three experiments, as hematocrit is one of the major hematological parameters used to measure the iron status of pigs and has a strong positive correlation with hemoglobin levels (Heidbüchel *et al.*, 2019). It has also been reported that hematocrit is possibly a better indicator of the iron status of weaning pigs, as it responds to the weight of the piglet more pronouncedly than hemoglobin (Joliff and Mahan, 2011). However, further research is needed to include more hematological parameters to clearly understand the response of pigs to various iron injection strategies.

In Experiment 1, the effect of the timing of the second iron injection to suckling pigs before weaning was evaluated. To demonstrate the effects clearly, this study used slightly lower doses of iron for pigs than the common dose used in the practical situation (200 mg of iron), which were 150 mg of iron at study initiation and 100 mg of iron for the second dose. In this experiment, the piglets receiving the second dose of iron at 14 days before weaning slightly increased body weight at 2 weeks postweaning and their postweaning growth rate compared with those receiving only 150 mg of iron at birth or the second dose of iron at 7 days before weaning. The preweaning growth performance did not differ among treatments, which agrees with previous studies (Bruininx *et al.*, 2000; Williams *et al.*, 2020; Albers *et al.*, 2022).

Table 6. Hematocrit levels (%) in pre- and postweaning periods in Experiment 3.

	Treatment[1]				P-value[2]		
	Fe100	**Fe200**	**BW200**	**SEM**	**1 vs 2**	**1 vs 3**	**2 vs 3**
Hematocrit levels, %							
0 d of exp.	23.08	25.81	24.56	1.27	0.13	0.38	0.41
10 d of exp.	34.11	36.56	36.19	1.76	0.05	0.08	0.70
17 d of exp.	31.71	33.81	38.44	2.07	0.49	0.06	0.15
24 d of exp.(wean)[3]	31.25	33.63	34.98	1.91	0.23	0.09	0.47
31 d of exp.[4]	30.97	35.57	37.07	0.99	0.01	0.01	0.13
38 d of exp.[5]	30.66	30.32	29.57	1.44	0.80	0.42	0.56

[1] 1) Fe100: 100 mg iron at d 0 of experiment (d 3 to 4 of age), 2) Fe200: 200 mg Fe at d 0 of experiment, and 3) BW200: 125 mg Fe per kg body weight at d 0 of experiment targeting 200 mg of iron injection for pigs weighing an average of 1.6 kg.
[2] n=8 per treatment.
[3] Weaning day.
[4] d 7 postweaning.
[5] d 14 postweaning.

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Similar results were also reported by Chevalier *et al.* (2023) in which the second iron injection at d 6-8 of age before weaning improved hematological parameters and postweaning growth performance of piglets but not preweaning growth. Hematocrit levels were greater in second iron injection treatments than the control treatment from 7 days after the second dose until the end of the experiment. The greater hematocrit values were observed in the Iron14 treatment than the Iron7 treatment at d 17 of experiment due to the earlier second dose in the Iron14 treatment. Our previous studies (Albers *et al.*, 2022) also reported that hematocrit levels increased with the second iron injection before weaning. As piglets are growing quickly during the suckling period and have a longer lactation period, one 200 mg iron injection may not be sufficient to meet their iron needs until weaning (Perri *et al.*, 2016), and an additional iron injection may be needed for pigs (Albers *et al.*, 2022; Chevalier *et al.*, 2023). However, Chevalier *et al.* (2021) reported that it could take several days for the injected iron to be involved in iron metabolism for hemoglobin synthesis. Williams *et al.* (2021) reported a quadratic response of iron injection timing to suckling piglets in hemoglobin and hematocrit levels in which the levels at d 12 of age were the highest when the piglets were injected with iron at d 4 of age, whereas those were lower when the piglets were injected with iron at d 6 to 10 of age. These results indicated that the full response of pigs to the injected iron for hemoglobin and hematocrit levels could take about 6-8 days from the iron administration. According to the results of the current study, the second iron injection may need to be performed in the early suckling period, and the effect of the second iron injection on growth may be observed after weaning, although the improvement in hematological parameters is likely to happen earlier than the performance response. However, a high level of iron injection to pigs could cause oxidative stress as it could result in increased free iron circulating in the blood and induce free radical formation, when excessive iron exists in the body over the capacity of transferrin (Szudzik *et al.*, 2018). Moreover, an additional iron injection before weaning requires more labor in swine production. Therefore, further studies are necessary to investigate how early the second iron injection can be performed to improve postweaning performance without potential oxidative stress and damage in pigs and reduce necessary labor.

In Experiment 2, the effect of split iron injection on suckling pigs was evaluated. There was no difference in preweaning growth performance. At the end of the experiment, the pigs receiving split iron injection had a greater body weight than the control pigs (one 100 mg iron injection at birth), while the pigs receiving one 200 mg of iron injection had intermediate values, which indicates that split iron injection may be more effective to improve postweaning pig growth compared with one high-dose of iron injection, as the postweaning growth rate tended to be greater in the pigs receiving split iron injection than the pigs receiving one high-dose. Chen *et al.* (2019) reported no difference in growth performance until d 15 of age when pigs were injected with iron in a single dose or split doses, whereas piglets receiving the split iron injection at d 3 and 14 of age with 40 mg of iron per kg body weight resulted in greater body weight at d 20 to 30 of age than those receiving one injection with 150 mg of iron per kg body weight, which agrees with the current study. A single 200 mg iron injection resulted in a slightly greater hematocrit level at the end of the experiment than split iron, which indicates that one high dose of iron may maintain greater hematocrit values for a longer period, although split iron injection may be more beneficial for pigs to improve postweaning growth performance. In addition, a single 200 mg iron injection at birth showed greater hematocrit levels at d 9 of experiment than the other 2 treatments receiving 100 mg iron injection at birth, as expected since hematocrit levels are positively correlated with the level of iron injected to newborn piglets (Williams *et al.*, 2020; Chevalier *et al.*, 2021; Albers *et al.*, 2022). As an excess of iron could be toxic to piglets (Szudzik *et al.*, 2018), split iron injection could reduce the degree of oxidative stress that pigs may face after iron injection. Chen *et al.* (2019) reported that a single high dose of iron injection at 150 mg iron per kg body weight resulted in oxidative stress but not oxidative injury or an inflammatory response in piglets. When pigs become larger in the suckling period, they require more iron due to increased blood volume and hemoglobin requirement (Friendship *et al.*, 2021) and have greater total iron binding capacity (Perri *et al.*, 2016). Therefore, split iron injection can be a useful strategy for iron injection to suckling pigs to reduce oxidative stress while improving postweaning growth performance.

In Experiment 3, the effect of body weight-based iron injection to suckling pigs was evaluated. There was no difference in pre- and postweaning growth performance among treatments, although pigs receiving only 100 mg of iron at birth had lower hematocrit levels until the first week postweaning compared with those receiving greater levels of iron at birth. Interestingly, only pigs receiving the iron injection based on their body weight had increased hematocrit levels greater than the control pigs during the suckling period, which indicates that a body weight-based iron injection targeting 125 mg of iron per kg body weight for pigs weighing an average of 1.6 kg could increase hematocrit levels more efficiently than a 200 mg of iron injection at birth regardless of body weight. Joliff and Mahan (2011) reported that heavier piglets at weaning have lower hemoglobin levels than lighter piglets, which indicates that heavier piglets at weaning are more susceptible to iron deficiency anemia after weaning, and larger piglets may need more iron for hemoglobin synthesis to support greater blood volume (Friendship *et al.*, 2021). However, a high level of iron injection could be more toxic to smaller piglets when injected with iron regardless of body size due to their lower total iron binding capacity as Perri *et al.* (2016) reported that small piglets (5.2 kg) at weaning (21 d of age) had lower total iron binding capacity than large piglets (7.5 kg). Therefore, body weight-based iron injection could reduce the potential toxic response and oxidative stress for smaller pigs and provide more iron for heavier piglets to reduce the potential risk of iron deficiency anemia.

In conclusion, the results of these three experiments provide scientific insights into the effects of different iron injection strategies on piglet growth performance and hematocrit levels during both pre- and post-weaning periods. The findings suggest that a second iron injection, particularly when given earlier than later before weaning, could have positive effects on postweaning growth performance. Split iron injection to pigs may be beneficial to improve postweaning growth performance compared with a high level of iron injection at birth. Although there was no difference in growth performance, body weight-based iron injection could potentially increase hematocrit levels greater than an iron injection regardless of body size. These findings contribute to our understanding of the optimal timing and dosage of iron supplementation for suckling piglets. The split and body weight-based iron injection strategies showed potential benefits in maintaining piglet health and minimizing the potential harm associated with excessive iron administration. However, it is important to consider factors such as the specific piglet population, the iron product used, weaning age, and management practices when implementing such strategies. Further studies are needed to demonstrate the potential risk of a high dose of iron injection on oxidative stress in pigs to safely apply these strategies to swine production.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest regarding the work presented in this report.

Author contributions

GL, CML, ANR, and YDJ were responsible for the design and conception of the study, collecting data, writing, reviewing, and critical reading of the paper. YDJ was responsible for administering the project and editing the paper.

Use of artificial intelligence (AI)

No AI or AI-assisted technologies were used during the preparation of this work.

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