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Effects of increasing Fe dosage in newborn pigs on suckling and subsequent nursery performance and hematological and immunological criteria

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Abstract

A total of 336 newborn pigs (DNA 241 × 600, initially 1.75 ± 0.05 kg bodyweight [BW]) from 28 litters were used in a 63-d study evaluating the effects of increasing injectable Fe dose on suckling and subsequent nursery pig performance and blood Fe status. GleptoForte (Ceva Animal Health, LLC, Lenexa, KS) contains gleptoferron which is an Fe macromolecule complex that is commercially used as an injectable Fe source for suckling piglets. On the day of processing (day 3 after birth), all piglets were weighed and 6 barrows and 6 gilts per litter were allotted within sex to 1 of 6 treatments in a completely randomized design. Treatments consisted of a negative control receiving no Fe injection and increasing injectable Fe to achieve either 50, 100, 150, 200 mg, or 200 mg plus a 100 mg injection on day 11 after birth. Pigs were weaned (~21 d of age) and allotted to nursery pens based on BW and corresponding treatment in a completely randomized design. During lactation, increasing injectable Fe up to 100 mg improved (quadratic; $P < 0.05$) average daily gain (ADG) and day 21 BW with no further improvement thereafter. There was no evidence of differences ($P > 0.10$) observed between the 200 mg and 200 mg + 100 mg treatments for growth. For the nursery period, increasing Fe dosage increased (linear; $P < 0.05$) ADG, average daily feed intake, and day 42 BW. There was no evidence of differences ($P > 0.10$) between the 200 mg and 200 mg + 100 mg treatments for nursery growth. For blood criteria, significant treatment × day interactions ($P = 0.001$) were observed for hemoglobin (Hb) and hematocrit (Hct). The interactions occurred because pigs that had <150 mg of injectable Fe had decreased values to day 21 and then increased to day 63 while pigs with 150 or 200 mg of injectable Fe had increased values to day 21 then stayed relatively constant to day 63. In summary, piglet performance during lactation was maximized at 100 mg while nursery growth performance and blood Fe status were maximized with a 200 mg Fe injection at processing. Providing an additional 100 mg of Fe on day 11 of age increased Hb, and Hct values at weaning and 14 d into the nursery but did not provide a growth performance benefit in lactation or nursery. These results indicate that providing 200 mg of injectable Fe provided from GleptoForte is sufficient to optimize lactation and subsequent nursery growth performance and blood Fe status.

Key words: Fe, gleptoferron, growth performance, nursery

Abbreviations

ADFI	average daily feed intake
ADG	average daily gain
BIC	Bayesian information criterion
BW	body weight
CP	crude protein
ELISA	enzyme-linked immunosorbent assay
$FeSO_4$	ferrous sulfate
G:F	gain-to-feed ratio
Hb	hemoglobin
Hct	hematocrit
Hp	haptoglobin
IM	intramuscular
IFN-γ	interferon-gamma
LPS	lipopolysaccharide
PHA	phytohemagglutinin
TIBC	total iron binding capacity
TNF-α	tumor necrosis factor-alpha

Introduction

Iron is an essential micronutrient involved in numerous biochemical processes such as electron transfer reactions, gene regulation, binding and transport of oxygen, and regulation of cell growth to maintain homeostasis within the body (Beard, 2001). Compared to other microminerals that are regulated through excretion, the maintenance of whole-body Fe homeostasis is through regulation of Fe absorption (Hallberg and Hulthén, 2000). In young swine, inefficient absorption of Fe reduces the number of circulating red blood cells resulting in anemia and poor growth performance (Kim et al., 2017). Newborn pigs are more susceptible to and develop Fe deficiency in the first week of life due to small Fe storages at birth, low levels of available Fe in sow colostrum, and the rapid growth rate that occurs during this period of a pig's life (Kegley et al., 2002). Because of this, an Fe injection within the first week of birth is commonly used in the swine industry to prevent Fe deficiency.

The negative consequences of no supplemental Fe injection during the first week of a young pig's life is well established. The absence of an Fe injection within this period results in decreased bodyweight (BW) and reduced Fe status at weaning (Peters and Mahan, 2008). Although the swine industry standard is a 200-mg Fe injection, research has been conducted to determine whether an extra injection of Fe later in lactation improves growth performance and Fe status. Joliff and Mahan (2011) determined that an extra 100 mg of Fe from iron dextran at day 10 of age can improve Fe status at weaning and initial postweaning performance. Although Lipinski et al. (2010) determined that a 100 mg injection of Fe from iron dextran in a single dose can reduce the bioavailability of Fe by increasing the expression of hepcidin which suppresses serum Fe circulation within the body and leads to inadequate development of red blood cells compared with injecting 40 mg of Fe at 2 different times.

GleptoForte (Ceva Animal Health, LLC, Lenexa, KS) is an injectable Fe source that contains gleptoferron. Gleptoferron is a macromolecule complex that has the potential for increased bioavailability which could allow for improved Fe status at weaning and improved growth performance. Research is not available that describes the optimal dosage of Fe from gleptoferron that supports maximum pre- and postweaning growth performance and Fe status. Therefore, the objective of this study was to determine the effects of increasing injectable Fe in newborn pigs on suckling and subsequent nursery performance and hematological and immunological 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

Lactation

A total of 336 newborn pigs (DNA 241 × 600, initially 1.75 ± 0.05 kg BW) from 28 litters were used in a 63-d study. The number of pigs per sow were equalized on each day of farrowing. On the day of processing (3 d after birth), all piglets were weighed, and 6 barrows and 5 gilts were allotted to 1 of 6 treatments in a completely randomized design. There was 1 barrow and 1 gilt per treatment for each sow. The six treatments consisted of a negative control receiving no injectable Fe or 50, 100, 150, or 200 mg Fe (Ceva Animal Health, LLC) provided in one injection with a 20-gauge, 1.27 cm needle (Ideal D3, Ideal Instruments, Neogen Animal Safety, Lexington, KY) in a 3-mL regular tip syringe (Monoject, Covidien, Dublin, Republic of Ireland), or a treatment with 200 mg provided on day 3 plus another 100 mg injection on day 11. Each 1 mL of injectable solution contained 200 mg of Fe, thus injection dosage was 0, 0.25, 0.50, 0.75, 1.0, or 1.0 mL plus 0.50 mL injection for each treatment, respectively. For practical delivery of the different doses of Fe, the volume of injectable solution was changed. It is not believed that this change in injection volume would impact the outcomes measured, rather it is believed that any observed differences would be due to the different Fe dosages. Piglets were weighed at processing (day 3), day 11, and weaning (day 21) to calculate average daily gain (ADG) during lactation. Creep feed was not offered to suckling pigs.

Nursery

Pigs were weaned at ~21 d of age and allotted to pens based on BW and previous Fe treatment in a completely randomized design with 5 or 6 pigs per pen and 10 pens per treatment. Each pen (1.52 × 1.52 m) had metal tribar flooring, one 4-hole self-feeder, and a nipple waterer to provide ad libitum access to feed and water. Pigs and feeders were weighed on days 10, 17, 24, 31, and 42 to determine ADG, average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

Diet preparation

All diets were corn–soybean meal based (Table 1). Common nursery diets were fed to all pigs in all nursery phases. Phase 1 diet was prepared at a commercial feed mill (Hubbard Feeds, Inc., Beloit, KS). Phase 1 diet contained specialty protein ingredients and was fed in pellet form. Phases 2 and 3 diets were prepared at the Kansas State University O.H. Kruse Feed Technology and Innovation Center located in Manhattan, KS. Phases 2 and 3 diets contained 55 mg/kg of carbadox (Mecadox, Phibro Animal Health Co., Stamford, CT) and were fed in meal form. All diets contained 110 mg/kg Fe from ferrous sulfate ($FeSO_4$) provided by the trace mineral premix and were formulated according to the Nutrient Requirements of Swine (NRC, 2012) to be at or above the pigs' daily nutrient requirements as not to limit growth performance.

Table 1. Nursery diet composition (as-fed basis)[1]

Item	Phase 1	Phase 2	Phase 3
Ingredient, %			
Corn	32.18	50.68	61.85
Soybean meal, 48% CP	20.29	29.62	33.75
Corn DDGS, 6–9% oil[2]	5.00	—	—
Enzymatically processed soy protein[3]	7.50	5.00	—
Fish meal	4.00	—	—
Choice white grease	3.00	—	—
Limestone	0.75	1.05	0.95
Monocalcium phosphate, 21%	0.70	1.05	1.15
Sodium chloride	0.30	0.30	0.35
L-Lysine hydrochloric acid	0.23	0.30	0.30
DL-Methionine	0.15	0.18	0.12
L-Threonine	0.09	0.15	0.12
Trace mineral premix[4]	0.15	0.15	0.15
Vitamin premix[5]	0.25	0.25	0.25
Choline chloride	0.04	—	—
Phytase[6]	—	0.02	0.02
Zinc oxide	0.39	0.25	—
Antimicrobial[7]	—	1.00	1.00
Total	100	100	100
Calculated analysis			
Standardized ileal digestible (SID) AA, %			
Lysine	1.40	1.35	1.24
Methionine:lysine	35	35	33
Methionine and cysteine:lysine	58	58	57
Threonine:lysine	63	66	63
Tryptophan:lysine	19	19	18.7
Valine:lysine	69	67	68
Total lysine, %	1.55	1.49	1.39
Metabolizable energy, kcal/kg	3,337	3,127	3,112
Net energy, kcal/kg	2,480	2,292	2,283
STTD P[8], %	0.52	0.52	0.48
Analyzed composition[9]			
Dry Matter, %	92.0	90.8	89.3
Crude Protein, %	21.5	23.4	20.9
Calcium, %	0.90	0.96	0.97
Phosphorous, %	0.70	0.67	0.66
Fe, mg/kg	420	273	255

[1]Phase 1 diets from days 0 to 10 (~5.7 to 6.2 kg), Phase 2 diets fed from days 10 to 24 (~6.2 to 11.1 kg), and Phase 3 diets fed from days 24 to 42 (~11.1 to 21.7 kg).
[2]Dried distillers grains with solubles.
[3]HP 300, Hamlet Protein, Inc., Findlay, OH.
[4]Provided per kilogram of premix: 22 g Mn from manganese oxide; 73 g Fe from iron sulfate; 73 g Zn from zinc sulfate; 11 g Cu from copper sulfate; 198 mg I from calcium iodate; and 198 mg Se from sodium selenite.
[5]Provided per kilogram of premix: 3,527,360 IU vitamin A; 881,840 IU vitamin D3; 17,637 IU vitamin E; 3,307 mg riboflavin; 1,764 mg menadione; 11,023 mg pantothenic acid; 33,069 mg niacin; and 15.4 mg vitamin B12.
[6]HiPhos 2700 (DSM Nutritional Products, Inc., Parsippany, NJ), provided 406.3 phytase units (FTU)/kg and an estimated release of 0.10% available P.
[7]Carbadox (Mecadox-2.5; Phibro Animal Health, Teaneck, NJ).
[8]Standardized total tract digestible phosphorous.
[9]Complete diet samples were obtained from each dietary phase directly at the feeder. Samples of diets were then submitted to Ward Laboratories, Inc. for analysis.

Chemical analysis

Six samples of complete diet per dietary phase were collected directly from feeders. The six samples were pooled, subsampled, and sent to a commercial laboratory (Ward Laboratories, Inc., Kearney, NE) for analysis of dry matter (AOAC 935.29, 2012), crude protein (CP; AOAC 990.03, 2012), Ca (AOAC 965.14/985.01, 2012), P (AOAC 965.17/985.01, 2012), and Fe (AOAC 999.11, 2012; Table 2).

Blood analysis

Blood samples were collected via jugular venipuncture in 5-mL ethylenediaminetetraacetic acid and whole blood (Monoject, Covidien) tubes using 22-gauge, 2.54 cm needles from each barrow per treatment per litter on 3, 11, 21, 35, and 63 d after birth. Hematological criteria measured included: hemoglobin (Hb) and hematocrit (Hct) using an ADVIA 2021i Hematology System (Siemens Healthcare Diagnostics, Tarrytown, NY) and serum Fe and total iron-binding capacity (TIBC) using a COBAS C501 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Blood samples were processed at the Kansas State University Veterinary Diagnostic Lab, Manhattan, KS.

Immunological analysis

Blood samples were collected via jugular venipuncture in 10-mL heparinized (159 USP units of Na heparin) Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) using 22-gauge, 2.54 cm needles from 1 barrow per treatment per litter 21 d after birth. Immunological criteria measured included: Haptoglobin (Hp), and functional assays to measure the ex vivo activity of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and plasma bactericidal effects. Haptoglobin was measured using colorimetric method based on peroxidase activity (Cooke and Arthington, 2013). TNF-α response was measured by subjecting blood samples to 800 μL of lipopolysaccharide (LPS) and placing in 96-well enzyme-linked immunosorbent assay (ELISA) plates coated with anti-swine TNF-α polyconal antibody (Kingfisher, Biotech, Inc., St. Paul, MN) and absorbance was measured using a microtech spectrometer (BioTek EON, Winooski, VT). Interferon-gamma response was measured by subjecting blood samples to 100 μL of phytohemagglutinin (PHA) and placing in 96-well ELISA plates coated with IFN-γ (Kingfisher, Biotech, Inc.) and absorbance measured using a microtech spectrometer (BioTek EON). Plasma blood killing was measured by plating a 1:4 dilution of blood plasma: *Escherichia coli* 51813 on trypticase soy agar plates (Thomas Scientific, Inc., Swedesboro, NJ) and allowing to incubate at 37.5 °C overnight. Plates were then counted for presence of *E. coli* infected cells to determine plasma bactericidal activity as previously described by Moisa et al. (2019).

Fecal and water analysis

Fecal samples from 8 sows were collected on days 3, 11, and 21 of the trial and pooled into a single sample, and then were submitted for duplicate analysis of Fe content (Experiment Station Chemical Laboratories, University of Missouri-Columbia, Missouri). Water samples from 6 different lactation crates and 6 different nursery pens were collected on each weigh day and pooled into a single sample and were then submitted for duplicate analysis of Fe content (Ward Laboratories, Inc.).

Statistical analysis

Growth data and immunological criteria were measured using the GLIMMIX procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) with individual pig as the experimental unit. Suckling piglet growth data and immunological criteria were analyzed as a completely randomized design. Crate and sex served as random effects in the growth model and crate served as a random effect in the immunological criteria model. Nursery growth data were

Table 2. Effects of injectable Fe dosage on preweaning pig performance[1]

	Fe, mg[2]							P <		
Item	0	50	100	150	200	200 + 100[3]	SEM	Linear[4]	Quadratic[5]	200 vs. 200 + 100[6]
BW, kg										
Day 3[7]	1.7	1.7	1.7	1.8	1.7	1.8	0.05	0.793	0.943	0.556
Day 11[8]	3.3	3.6	3.5	3.6	3.5	3.5	0.01	0.012	0.018	0.702
Day 21	4.7	5.7	5.9	5.8	5.8	5.7	0.15	0.001	0.001	0.800
ADG, g										
Days 3 to 11	192	230	228	227	226	218	8.2	0.002	0.002	0.409
Days 11 to 21	154	223	244	229	234	233	8.3	0.001	0.001	0.881
Days 3 to 21	171	226	237	228	230	227	7.3	0.001	0.001	0.605

[1]A total of 336 suckling pigs (DNA 241 × 600) from 28 litters were used with 12 pigs per sow and 2 replications of treatment within sow.
[2]Fe (Gleptoforte, Ceva Animal Health, LLC) dosage administered 3 d after farrowing.
[3]Pigs were administered 200 mg at 3 d after farrowing and 100 mg 11 d after farrowing.
[4]Linear comparison of 0 to 200 mg dosage.
[5]Quadratic comparison of 0 to 200 mg dosage.
[6]Pairwise comparison between mean of 200 and 200 + 100 mg treatments.
[7]Represents 3 d after birth.
[8]Represents 11 d after birth.

analyzed as a completely randomized design with pen as the experimental unit and room as a random effect. Treatment served as the fixed effect in both growth and immunological criteria models. Hematological criteria from suckling and nursery pigs were analyzed as a repeated measure using crate as a random effect. The Bayesian information criterion (BIC) was used to determine best fit, with a lower number indicating an improved fit. A decrease in BIC >2 among models for a hematological criterion was considered a significant improvement in fit (Gonçalves et al., 2016). For the hematological criteria model, main effects of treatment and day, as well as their interaction, were evaluated. For all models, preplanned contrasts were utilized to evaluate linear and quadratic effects of Fe dosage from 0 to 200 mg and a pairwise comparison of the 200 mg vs. 200 + 100 mg treatments. Results were considered significant at $P \leq 0.05$ and marginally significant at $P > 0.05$ and $P \leq 0.10$.

Results

Chemical analysis

Water collected from lactation crates averaged 1.22, 0.90, and 0.24 µg/mL Fe on days 3, 11, and 21, respectively. Results of water analysis collected from nursery pens averaged 0.03, 0.01, and 0.03 µg/mL Fe on days 0, 14, and 42, respectively. Results of fecal analysis collected from sows during lactation averaged 1,735, 1,610, and 1,140 mg/kg Fe (as-is basis) on days 3, 11, and 21, respectively. Analyzed Fe content in the three dietary nursery phases ranged from 255 to 420 mg/kg.

Lactation growth performance

From days 3 to 11 after birth, ADG of piglets improved (quadratic; $P = 0.002$) with increasing injectable Fe up to 50 mg with no further improvement thereafter (Table 2). Day 11 BW of piglets increased (quadratic; $P = 0.018$) with increasing injectable Fe up to 50 mg with no further improvement thereafter. From days 11 to 21 (weaning), ADG of piglets increased (quadratic; $P = 0.001$) with increasing dosage of Fe up to 100 mg with no further improvement thereafter. Overall, ADG and day 21 BW increased (quadratic; $P = 0.001$) with increasing injectable Fe dosage up to 100 mg with no improvement observed thereafter. Furthermore, there was no evidence for differences ($P > 0.10$) in preweaning performance between the 200 mg and 200 mg + 100 mg injectable Fe treatments.

Nursery growth performance

From days 0 to 10 after weaning, increasing injectable Fe administered postfarrowing improved (linear; $P < 0.05$) ADG, ADFI, and G:F (Table 3). Furthermore, increasing injectable Fe up to 100 mg improved (quadratic; $P = 0.001$) day 10 BW with little improvement thereafter. From days 10 to 24, increasing injectable Fe administered postfarrowing improved (quadratic; $P < 0.05$) ADG and G:F. Increasing injectable Fe up to 150 mg also improved (quadratic; $P = 0.009$) day 24 BW with no further improvement with increased dosage. From days 0 to 24, increasing injectable Fe improved (linear; $P < 0.05$) ADG and ADFI. Furthermore, increasing injectable Fe up to 150 mg improved (quadratic; $P = 0.017$) G:F with no further improvement as dosage increased thereafter. From days 24 to 42, increasing injectable Fe improved (linear; $P < 0.05$) ADG and day 42 ending BW, and marginal significance for improved (linear; $P = 0.071$) ADFI was observed.

Overall (days 0 to 42), increasing injectable Fe improved (linear; $P = 0.05$) ADG and ADFI. Furthermore, increasing injectable Fe up to 150 mg improved (quadratic; $P = 0.011$) G:F with a worsening G:F observed when 200 mg was administered to pigs. There was no evidence of difference in growth performance ($P > 0.10$) between the 200 mg and the 200 + 100 mg injectable Fe treatments during any phase or overall.

Hematological criteria

As expected, there was no evidence of difference ($P > 0.10$) observed for any hematological criteria measured on day 3 prior to the Fe injection (Table 4). For Hb, a significant treatment × day interaction ($P = 0.001$) was observed. The interaction occurred because pigs receiving <150 mg of injectable Fe had decreased Hb values to day 21 and then increased to day 63 with the feeding of common diets. Conversely, for pigs receiving the 150 or 200 mg injections, Hb values increased to day 21 and then stayed relatively constant to day 63. Hb values increased (quadratic; $P = 0.001$) on days 11 and 21, and (linear; $P = 0.001$) day 35 with the 0 mg treatment having the lowest Hb values

Table 3. Effects of Fe dosage on nursery pig performance[1]

	Fe, mg[2]							P <		
Item	0	50	100	150	200	200 + 100[3]	SEM	Linear[4]	Quadratic[5]	200 vs. 200 + 100[6]
BW, kg										
Day 0	4.9	5.7	5.9	5.8	5.8	5.8	0.08	0.001	0.001	0.997
Day 10	5.2	5.9	6.3	6.4	6.4	6.6	0.27	0.001	0.001	0.339
Day 24	9.3	10.6	11.2	11.7	11.6	12.1	0.27	0.001	0.009	0.277
Day 42	19.4	21.1	21.6	22.7	22.9	22.6	0.53	0.001	0.209	0.730
Days 0 to 10										
ADG, g	32	23	43	57	63	80	23.4	0.005	0.524	0.264
ADFI, g	109	104	115	132	142	140	17.5	0.009	0.375	0.881
G:F	0.301	0.085	0.344	0.402	0.425	0.506	0.1409	0.013	0.287	0.415
Days 10 to 24										
ADG, g	283	327	354	378	364	376	15.6	0.001	0.036	0.563
ADFI, g	510	507	505	542	536	564	22.9	0.198	0.674	0.353
G:F	0.561	0.645	0.704	0.700	0.680	0.681	0.0275	0.001	0.011	0.975
Days 0 to 24										
ADG, g	176	200	224	245	238	251	11.3	0.001	0.136	0.430
ADFI, g	340	338	342	371	371	384	14.8	0.048	0.609	0.524
G:F	0.524	0.587	0.658	0.661	0.641	0.658	0.0263	0.001	0.017	0.636
Days 24 to 42										
ADG, g	535	586	574	593	617	587	19.6	0.003	0.704	0.246
ADFI, g	882	916	917	921	989	936	45.6	0.071	0.622	0.322
G:F	0.608	0.643	0.628	0.648	0.628	0.633	0.0141	0.323	0.157	0.787
Days 0 to 42										
ADG, g	326	364	374	392	397	392	13.8	0.001	0.212	0.794
ADFI, g	566	583	588	604	629	617	23.3	0.029	0.740	0.666
G:F	0.577	0.624	0.637	0.651	0.632	0.640	0.0132	0.002	0.011	0.698

[1]A total of 336 nursery pigs (DNA 241 × 600) from 28 litters were used with 5 or 6 pigs per pen and 10 replications per treatment. Common diets were fed throughout the nursery phase and contained 110 mg/kg added Fe from $FeSO_4$ provided from the trace mineral premix.
[2]Fe (Gleptoforte, Ceva Animal Health, LLC) dosage administered 3 d after farrowing.
[3]Pigs were administered 200 mg at 3 d after farrowing and 100 mg 11 d after farrowing.
[4]Linear comparison of 0 to 200 mg dosage.
[5]Quadratic comparison of 0 to 200 mg dosage.
[6]Pairwise comparison between mean of 200 and 200 + 100 mg treatments.

and the 200 mg treatment having the greatest values. There was no evidence of difference ($P > 0.10$) observed for Hb values measured on day 63. On days 21 and 35, the 200 mg + 100 mg treatment led to an increase ($P < 0.05$) in Hb values compared with the 200-mg treatment.

A significant treatment × day interaction ($P = 0.001$) was observed for Hct values. The interaction was the result of Hct values decreasing for pigs receiving no injectable Fe or 50 mg of injectable Fe to day 21 and then increasing to day 63 with the feeding of common diets. For pigs receiving 100 mg of injectable Fe, Hct values increased to day 11 and then decreased to day 21. From days 21 to 63, Hct values continued to increase with the feeding of common diets. For pigs receiving 150 or 200 mg of injectable Fe, Hct values increased to day 21 and then stayed relatively constant to day 63. Increasing injectable Fe increased (quadratic; $P = 0.001$) Hct values on days 11, 21, and (linear; $P = 0.001$) day 35 with pigs receiving no Fe having the lowest Hct value and the 200 mg treatment having the greatest Hct value. There was no evidence of difference ($P > 0.10$) observed for Hct values measured on day 63. On day 21 and 35, the 200 mg + 100 mg treatment led to an increase ($P < 0.05$) in Hct values compared with the 200-mg treatment.

For serum Fe, a significant treatment × day interaction ($P = 0.01$) was observed. The interaction was the result of serum Fe values for pigs receiving <100 mg of injectable Fe staying relatively constant to day 21 and then increasing to day 63 with the feeding of common diets. Meanwhile, for pigs receiving 100 mg of injectable Fe, serum Fe values increased to day 11 and then decreased to day 21. From days 21 to 63, serum Fe values increased for these pigs with the feeding of common diets. For the 150 and 200 mg treatments, serum Fe values increased to day 11 and then decreased to day 21. Serum Fe values for pigs receiving these treatments increased from days 21 to 35 and then stayed relatively constant to day 63 with the feeding of common diets. Increasing injectable Fe increased serum Fe values on day 11 (linear; $P = 0.001$), day 21 (quadratic; $P = 0.002$), and day 35 (linear; $P = 0.001$) with the 0 mg treatment having the lowest serum Fe values and the 200 mg treatment having the greatest serum Fe values. There was no evidence of difference ($P > 0.10$) observed for serum Fe values measured on day 63. On day 21, the 200 mg + 100 mg treatment had an increase ($P = 0.030$) in serum Fe values compared with the 200-mg treatment.

A significant treatment × day interaction ($P = 0.01$) was observed for TIBC values. The interaction was the result of TIBC values increasing for all treatments from days 3 to 21 except for the 200 mg treatment staying constant from days 11 to 21. From days 21 to 35, TIBC values decreased for all treatments while pigs receiving 0 or 50 mg of injectable Fe continued to decrease to day 63 with the feeding of common diets. From days 35 to 63, pigs receiving 100 mg of injectable Fe had relatively constant TIBC while pigs receiving 150 or 200 mg of injected Fe increased with the feeding of common diets. Increasing injectable Fe decreased (quadratic; $P = 0.001$) TIBC values on day 11 and (linear; $P = 0.001$) on days 21 and 35 with the 0 mg treatment having the greatest

Table 4. Effects of Fe dosage on suckling and nursery pig hematological criteria[1]

	Fe, mg[2]							P <		
Item	0	50	100	150	200	200 + 100[3]	SEM	Linear[4]	Quadratic[5]	200 vs. 200 + 100[6]
Hb, g/dL[7]										
Day 3[8]	8.4	8.3	8.3	8.3	8.2	8.5	0.24	0.636	0.816	0.512
Day 11[9]	5.7	8.3	9.9	10.1	10.7	10.5	0.22	0.001	0.001	0.731
Day 21	4.6	6.8	9.3	11.3	12.0	12.8	0.22	0.001	0.001	0.012
Day 35	7.4	8.4	10.0	10.8	11.6	12.7	0.23	0.001	0.150	0.001
Day 63	12.0	11.8	12.0	12.1	12.0	12.4	0.24	0.610	0.789	0.287
Hct, %[7]										
Day 3	28.0	27.1	27.6	27.4	27.4	28.0	0.72	0.688	0.684	0.567
Day 11	20.0	29.2	34.4	35.8	36.5	36.2	0.71	0.001	0.001	0.782
Day 21	16.0	23.4	30.9	37.3	38.8	40.9	0.71	0.001	0.001	0.038
Day 35	26.4	30.0	33.6	35.5	37.2	40.6	0.72	0.001	0.072	0.001
Day 63	40.9	39.4	40.1	40.4	39.7	41.1	0.76	0.612	0.669	0.204
Serum Fe, µg/dL[7]										
Day 3	26	24	30	29	25	24	8.8	0.920	0.744	0.927
Day 11	19	29	101	149	162	157	8.7	0.001	0.558	0.675
Day 21	22	15	25	53	86	113	8.7	0.001	0.002	0.030
Day 35	88	99	121	150	138	147	9.4	0.001	0.267	0.481
Day 63	143	142	130	144	136	128	8.9	0.690	0.711	0.547
TIBC, µg/dL[7,10]										
Day 3[8]	252	248	216	236	242	223	19.9	0.594	0.324	0.507
Day 11	698	536	442	417	406	421	19.6	0.001	0.001	0.606
Day 21	726	667	519	479	415	398	19.7	0.001	0.174	0.546
Day 35	631	536	468	442	394	378	20.1	0.001	0.090	0.588
Day 63	500	495	478	496	495	490	22.4	0.896	0.607	0.883

[1]A total of 336 pigs (DNA 241 × 600) from 28 litters were used in a 63-d experiment with 12 pigs per sow and 2 replications of each treatment within sow. Pigs were weaned at 21 d and placed in pens with 5 or 6 pigs per pen and 10 replications per treatment. All barrows were bled at each of the timepoints to measure hematological criteria. Each timepoint represents days after farrowing. Days 3 and 11 represent timepoints in lactation and days 21, 35, and 63 represent timepoints in the nursery. Common diets were fed throughout the nursery phase and contained 110 mg/kg added Fe from $FeSO_4$ provided from the trace mineral premix.
[2]Fe (Gleptoforte, Ceva Animal Health, LLC) dosage administered 3 d after birth.
[3]Pigs were administered 200 mg at beginning of trial and 100 mg 11 d after farrowing.
[4]Linear comparison of 0 to 200 mg dosage.
[5]Quadratic comparison of 0 to 200 mg dosage.
[6]Pairwise comparison between mean of 200 and 200 + 100 mg treatments.
[7]Trt × day interaction ($P < 0.001$).
[8]Represents 3 d after birth. Blood was drawn prior to Fe injection.
[9]Represents 11 d after birth. Blood was drawn prior to Fe injection.
[10]Total Fe binding capacity.

TIBC values and the 200-mg treatment having the lowest TIBC values. There was no evidence of difference ($P > 0.10$) between the treatments on day 63. There was no evidence of difference ($P > 0.10$) between the 200 mg and 200 + 100 mg treatments at any of the collection timepoints for TIBC.

Immunological criteria

On day 21 after birth, there was no evidence of difference ($P > 0.10$) in Hp values with increasing levels of injectable Fe (Table 5). There was no evidence of difference ($P = 0.560$) between the 200 mg and 200 + 100 mg treatments on day 21. For LPS TNF-α, increasing levels of injectable Fe decreased (linear; $P = 0.017$) values with pigs receiving no injectable Fe having the greatest LPS TNF-α values and pigs receiving 200 mg of injectable Fe having the lowest LPS TNF-α values. There was no evidence of difference ($P = 0.225$) in LPS TNF-α values between the 200 mg and 200 + 100 mg treatments. There was no evidence of difference ($P > 0.10$) in PHA IFN-γ values amongst the treatments on day 21 after birth. Also, there was no evidence of difference ($P = 0.805$) between the 200 mg and 200 + 100 mg treatments. Increasing injectable Fe up to 100 mg decreased (quadratic; $P = 0.040$) plasma blood kill percentage with an increase in values thereafter up to 200 mg of injectable Fe. At weaning (day 21), the 200 mg treatment had a greater ($P = 0.029$) plasma blood kill percentage than the 200 + 100 mg treatment.

Discussion

Iron is transported to the developing fetus through endometrial secretion of the glycoprotein uteroferrin (Mahan and Vallet, 1997). The Fe of uteroferrin is used for Hb synthesis and is primarily stored in the liver (Ducsay et al., 1982). However, transport of uteroferrin across the maternoplacental barrier is limited and this causes suckling pigs to be born with inadequate Fe stores (Renegar et al., 1982). Furthermore, low amounts of Fe are provided from sow's colostrum and milk with each containing ~2.84 and 1.96 µg/mL, respectively (Hurley, 2015). Increasing Fe content and/or providing different sources in the sow's diet in an effort to increase Fe provided from colostrum and milk to improve Fe status or growth have shown to be inconclusive (Peters and Mahan et al., 2008; Novais et al., 2016). When growth requirements for suckling pigs are not met, anemia can occur

Table 5. Effects of Fe dosage on immune function criteria at weaning (21-d postfarrowing)[1]

	Fe, mg[2]							P <		
Item	0	50	100	150	200	200 + 100[3]	SEM	Linear[4]	Quadratic[5]	200 vs. 200 + 100[6]
Haptoglobin, μg/mL	73.6	75.8	63.6	65.3	60.6	52.1	8.52	0.141	0.809	0.560
LPS TNF-α, pg/mL	575	406	406	344	240	364	76.5	0.017	0.779	0.225
PHA IFN-γ, pg/mL	141	155	137	136	134	143	24.8	0.727	0.889	0.805
Plasma blood kill, %	60.3	49.8	45.8	52.3	51.4	39.1	5.07	0.115	0.040	0.029

[1]Blood samples were collected via jugular venipuncture from 1 barrow per treatment per litter (28 litters) on day 21 after farrowing. Immune function criteria were measured from 1 barrow per treatment per sow.
[2]Fe (Gleptoforte, Ceva Animal Health, LLC) dosage administered 3 d after farrowing.
[3]Pigs were administered 200 mg at beginning of trial and 100 mg 11 d after farrowing.
[4]Linear comparison of 0 to 200 mg dosage.
[5]Quadratic comparison of 0 to 200 mg dosage.
[6]Pairwise comparison between mean of 200 and 200 + 100 mg treatments.

(defined as Hb concentration ≤ 9 g/dL) and is characterized as hypochromic, microcytic anemia (Bhattarai et al., 2018). Because environmental sources of Fe that are available to suckling pigs during lactation are inadequate to meet the Fe growth requirement and prevent anemia, an exogenous source of Fe is needed.

Analysis of water samples indicated that there were low levels of iron present as would be expected considering the facility was supplied with a municipal water source. Sow fecal samples contained higher Fe concentrations compared with the water source or nursery diet concentrations, but are consistent with other reports (Barros et al., 2019). It is believed that acquisition of Fe and other minerals through ingestion of sow feces is minimal (Barros et al., 2019). Analyzed Fe content in phase 1 nursery diet was higher than formulated values, while phases 2 and 3 analyzed values were slightly lower than expected. However, all dietary Fe levels were well above the requirement of nursery pigs (NRC, 2012).

A single intramuscular (IM) injection of 200 mg of Fe is commonly used in the swine industry in an effort to prevent anemia and support growth. Researchers have shown that the absence of an Fe injection within the first week of a pig's life results in reduced lactation growth performance, weaning weight, and subsequent nursery growth performance similar to the current study (Peters and Mahan et al., 2008; Chevalier, 2019). It has been shown that in piglets not provided injectable Fe at birth, greater growth rate, hematocrit, and hemoglobin was achieved when piglets were fed a liquid milk-replacer with 21.3 mg/L Fe compared to a liquid milk-replacer with 2.72 mg/L Fe (Knight and Dilger, 2018). Thus, supplemental Fe is very important for young pigs and under commercial rearing conditions and is commonly provided as an Fe injection after birth to support lactation and subsequent nursery growth performance.

Previous studies have established the effectiveness of a single IM injection of 200 mg of Fe to support growth requirements (Pollmann et al., 1983; Yu et al., 2002; Morales et al., 2018). Although some research suggests that a single IM injection of 200 mg of Fe is insufficient to support growth requirements. This is due to faster growing or larger pigs being more at risk of exhibiting low Fe status at weaning and could potentially reduce subsequent growth performance (Bhattarai and Nielsen, 2015b; Almond et al., 2017; Gillespie, 2019). Perri et al. (2016) observed that pigs exhibiting low Fe status at weaning were 0.82 kg lighter 3 wk postweaning than pigs with normal Fe status. Furthermore, Van Gorp et al. (2012) suggest that a single IM injection of 200 mg of Fe would only support 4 kg of growth before weaning and estimated that 390 mg of Fe is needed to prevent the development of Fe deficiency before weaning. Bruinnix et al. (2000) observed that administration of 200 mg of Fe 3 d after birth plus an additional 200 mg of injected Fe 7 d before weaning provided no improvements in growth compared with a single injection of 200 mg of Fe 3 d after birth. Joliff and Mahan (2011) observed no improvement when providing 300 mg of Fe vs. 200 mg. The authors further evaluated the effects of administering 200 mg of Fe at birth plus a 100 mg injection of Fe 10 d after birth and found no evidence of difference in weaning weights, but marginal evidence of improvements in initial postweaning ADG compared with a single 200 mg injection of Fe at birth. Almond et al. (2017) observed that administration of a second injection of 150 mg of Fe ~5 to 7 d after birth provided inconsistent responses in subsequent postweaning growth compared with a single injection of 150 or 200 mg of Fe at 3 to 5 d of age. Chevalier et al. (2019) on the other hand observed that pigs receiving an injection of 150 mg of Fe 1 d after birth and an additional 150 mg injection of Fe 4 d before weaning exhibited increased nursery ADG and ending BW compared with pigs receiving a single injection of 150 mg of Fe 1 d after birth. The study herein observed no evidence of differences for preweaning or subsequent nursery growth performance when pigs were administered 200 mg of Fe 3 d after birth plus an additional 100 mg of Fe 11 d after birth compared with pigs receiving 200 mg of Fe alone. One possibility for these discrepancies is the varying timepoints and dosages in which the additional Fe is administered during lactation and warrants further investigation.

Hemoglobin concentration is one of the most widely used blood criteria measures to evaluate Fe deficiency and anemia in swine. Bhattarai and Nielsen (2015b) defined normal Fe as an Hb concentration > 11 g/dL, Fe deficiency as an Hb concentration >9 g/dL but ≤11 g/dL, and anemia as an Hb concentration ≤9 g/dL. The negative effects of no Fe injection after farrowing on Hb concentrations through weaning and subsequent nursery performance has been established (Peters and Mahan, 2008). Bhattarai and Nielsen (2015a) observed a positive association between Hb and ADG with an increase in 10 g Hb/L blood corresponding to a weight gain improvement of 17 g daily weight gain 3 weeks postweaning. Our results would agree in that a 200-mg injection of Fe 3 d after birth improves initial nursery performance. Also, our results agree with Kay et al. (1980) in that pigs receiving 100 mg of Fe after birth have similar performance to that of pigs receiving 200 mg of Fe up to weaning, but Hb

values at weaning are lower in the pigs receiving 100 mg of Fe compared with the pigs receiving 200 mg of Fe. Gentry et al. (1997) suggests that pigs with greater Hb status have improved energy retention compared with pigs with lower Hb status at weaning, but this needs to be explored further.

Along with Hb, Hct is a widely used blood criteria to monitor the Fe status of pigs. Several authors have defined the reference range for Hct indicating normal blood Fe status as Hct values > 30% and Fe deficiency as Hct values < 30% (Egeli et al., 1998; Perri et al., 2017). Similar to that of Pollmann et al. (1983) and Kegley et al. (2002), a 200-mg injection of Fe from gleptoferron resulted in greater Hct values at weaning and initially postweaning compared with pigs not receiving an Fe injection. As with Hb, performance of pigs receiving 100 mg of Fe after birth have similar growth performance to that of pigs receiving 200 mg of Fe after birth, but Hct values at weaning are greater in pigs receiving 200 mg of Fe after birth compared with pigs receiving only 100 mg of Fe after birth. Similar to that of Hb, Bhattarai and Nielsen (2015a) found a positive correlation between Hct values at weaning and improved growth rate initially postweaning in pigs. Our study would be in agreement with these results and shows the improved blood Fe status with a single injection of 200 mg of Fe.

Although Hb and Hct are normally used as indicators to determine Fe deficiency and anemia in young pigs, some researchers suggest that these blood criteria may underestimate the Fe requirement of piglets because the sensitivity and specificity of these criteria for diagnosis of Fe deficiency and anemia are low (Svoboda et al., 2008). Furthermore, the indices may not accurately indicate early Fe deficiency because erythrocytes have a slow turnover rate (Cook, 2005). Bhattarai and Nielsen (2015b) suggest that serum Fe and TIBC may be more suitable indicators to determine Fe deficiency as they are earlier indicators of erythropoietic activity in piglets than indicators such as Hb and Hct of mature erythrocytes. Total iron binding capacity is the measure of total serum transferrin and reveals the amount available for binding and transfer of Fe in the body. Limited reference values for serum Fe and TIBC are available in swine to determine pigs that are Fe deficient. Perri et al. (2017) observed that pigs with serum Fe values < 43.0 to 47.0 μmol/L and TIBC values < 121.0 to 125.0 μmol/L would be considered Fe deficient.

In the present study, pigs administered <150 mg of Fe 3 d after birth had serum Fe and TIBC values that would be considered Fe deficient at weaning according to Perri et al. (2017). This indicates that serum Fe and TIBC could possibly be used as indicators for Fe deficiency in suckling piglets and explains why these pigs experienced reduced nursery growth performance. Furthermore, research has consistently shown that a single injection of 200 mg of Fe after birth will increase serum Fe values at weaning (Pollman et al., 1983; Zhao et al., 2015; Morales et al., 2018). This would be in agreement with the current study as pigs injected with 200 mg of Fe 3 d after birth had greater serum Fe values at weaning and improved Fe status entering the nursery stage. The current study also observed that a single injection of 200 mg of Fe after birth decreased TIBC values at weaning, indicating that more serum transferrin was transporting Fe throughout the body and an improved blood Fe status. Research has shown that pigs receiving a single injection of 200 mg of Fe exhibit lower TIBC values at weaning and would agree with the results from the study herein (Pollman et al., 1983; Sperling et al., 2018). Morales et al. (2018) also observed that serum Fe decreased from days 14 to 21 in pigs injected with 200 mg of Fe after birth from either gleptoferron or Fe dextran, similar to the study herein.

Iron plays a vital role in immunity and is necessary for development of the immune system such as immune cell proliferation to generate a specific response to infection (Beard, 2001). Haptoglobin is an acute phase protein that is produced by the liver and binds to hemoglobin which prevents Fe loss and renal system damage and functions as an antioxidant with antibacterial activity and modulates the acute phase response (Wassell, 2000). As a function of its antioxidant activity, Hp prevents the generation of hydroxyl radicals and lipid peroxides that are produced by hemoglobin (Sauerwein et al., 2005). In swine, fetal hemoglobin is absent and there is no need for decomposing large amounts of hemoglobin. Therefore, Hp secretion is independent of hemolysis (Tautz and Kleihauer, 1972; Dobryszycka, 1997). This explains why in the current study no evidence of difference ($P > 0.10$) was observed for Hp values at weaning.

TNF-α and IFN-γ are critical proinflammatory cytokines in the host defense system and the absence or impairment of these cytokines severely weakens the host defense system against pathogens (Pfeffer, 2003). Both of these cytokines are released by the activation of immune cells in response to an infection. Yu et al. (2002) observed that in piglets challenged with endotoxin LPS the piglets that did not receive an Fe injection at birth had greater increases in TNF-α values than pigs injected with 200 mg of Fe. Our study agrees in that, at weaning, a decrease in TNF-α response following ex vivo stimulation with increasing Fe injection was observed. Furthermore, Li et al. (2016) observed that pigs fed diets high in Fe (520 mg/kg) had greater upregulation of duodenal TNF-α than pigs fed adequate Fe (120 mg/kg) or low Fe (20 mg/kg) diets, but had no evidence of difference in upregulation of IFN-γ. A better understanding is needed for the differences in regulation between IFN-γ and TNF-α in regard to Fe supplementation in swine.

The ability of the immune system of young swine to eliminate bacteria from the bloodstream is of importance. When the host goes into a nutritional deficiency to limit Fe in the serum or excess Fe is circulated, susceptibility to *E. coli* infection and proliferation is possible (Messenger and Barclay, 1983). Iron deficiency has been shown to negatively alter the bactericidal activity in humans (Chandra, 1973). Seip (2018) observed that in pigs challenged with enterotoxigenic *E. coli* receiving either a single injection of 100 or 200 mg of Fe 3 d after birth showed no evidence of difference in clinical signs or severity of symptoms. The current study observed that pigs not receiving an Fe injection had the highest ex vivo blood killing percentage of *E. coli*, but the blood killing increased with 150 and 200 mg injections of Fe. Furthermore, the pigs receiving an extra 100 mg dose of Fe had lower blood killing percentages compared with the pigs only receiving 200 mg of Fe. This could possibly be due to the excess Fe from the second injection allowing for proliferation of *E. coli* up to weaning.

In summary, this study has provided evidence that piglet growth performance during lactation was increased up to a 100 mg injection of Fe with no benefits observed with higher doses of Fe. However, postweaning growth performance was improved linearly by providing up to 200 mg of injectable Fe at processing. Blood Fe status pre- and postweaning was increased with a 200-mg injection of Fe. Providing an additional 100 mg of Fe on day 11 of age did not affect pre- or postweaning growth performance, but increased Hb, Hct, and serum Fe values at weaning and 14 d in the nursery. Furthermore, feeding diets that

are sufficient to meet the pig's Fe requirement restored blood Fe measurements in pigs that received low doses of supplemental Fe. Although blood Fe status is recaptured at the end of the nursery, performance of these pigs receiving the low Fe injection was still poorer than that of the pigs receiving the higher dosage.

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Conflict of interest statement

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