# Effects of Fe supplementation in newborn and nursery pigs on growth performance and hematological criteria

by

Hayden Ervin Williams

B.A., Wabash College, 2015
M.S., Kansas State University, 2017

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Animal Sciences and Industry
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2020

# Abstract

This dissertation consists of 4 chapters involving studies with administration of Fe dosage and the timing of Fe administration after birth in newborn pigs, source of Fe supplementation in nursery pigs, and feeding high levels of SID Trp:Lys ratios with ractopamine HCL in finishing pigs. Chapter 1 involved an experiment that used 336 newborn pigs to determine the effects of increasing injectable Fe dosage provided from gleptoferron after birth on suckling and subsequent nursery performance and blood criteria. The results suggest that providing 100 mg of Fe from gleptoferron is sufficient to support growth up to weaning but providing 200 mg of Fe from gleptoferron optimizes subsequent nursery growth performance and pre- and postweaning hematological criteria. The results further suggest providing an additional 100 mg of Fe from gleptoferron on d 11 of age result in no evidence for an effect on pre- or postweaning growth performance but increased hematological criteria at weaning and 14 d in the nursery. Chapter 2 involved two experiments that used 2,216 newborn pigs to determine the effects of age of newborn pigs receiving a Fe injection on suckling and subsequent nursery and growing-finishing growth performance and blood criteria. The results suggest that providing 200 mg of Fe from gleptoferron between d 1 and 7 after farrowing is sufficient for optimizing pre and post-weaning growth performance. The results further suggest that providing an additional 200 mg of Fe from gleptoferron 12 d after birth resulted in no evidence for an effect on preweaning growth performance but increased hematological criteria at weaning. Chapter 3 involved one experiment that used 140 Fe-deficient weanling pigs evaluating Fe sources ($FeSO_4$ or $FeCO_3$) and level (10 to 50 mg/kg) on nursery growth performance and hematological criteria. The results showed that the micronized form of $FeCO_3$ is a sufficient alternative source of Fe that can be added to nursery diets to meet post-weaning Fe requirements. The results also suggest that the Fe deficient

model utilized was sufficient in evaluating the availability of the 2 different Fe sources and their effects on nursery pig growth performance and hematological criteria. Chapter 4 had 1 experiment that used 1,791 finishing pigs to determine the effects of high SID Trp:Lys ratios in diets containing ractopamine HCL. The results suggested that increasing the SID Trp:Lys ratio above 20% that provided Trp intake above the NRC (2012) requirement of 4.1 g/d showed no evidence for an improvement in growth performance or carcass characteristics.

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# Table of Contents

# List of Tables

# Acknowledgements

This dissertation would not have been possible without the help and support of several individuals. I would like to thank my major professors, Dr. Joel DeRouchey and Dr. Jason Woodworth, for their support, knowledge, and patience throughout my doctoral program. Furthermore, I am extremely appreciative to the members of my committee, Drs. Dritz, Goodband, and Tokach, for their instruction, time dedication, and support throughout my program. The training and education I have received at K-State is next to none and feel confident in my preparation for my future career. Moreover, I am thankful for the chance all of these individuals gave to me to be a part of the K-State family and am ever grateful for the opportunity to work with all of these individuals.

I am fortunate to have been a part of a unique team atmosphere here at K-State and am ever grateful for the graduate students of the swine nutrition team who came before me and established this family atmosphere and for the current members who made this journey possible.

I would also like to thank the KSU swine farm, lab, feed mill, and several industry partners for their help and collaboration on my projects, Dr. Kyle Coble, Amanda Gerhart, Brittany Carrender, and JBs live pork, Dr. Andrew Holtcamp and CEVA Animal Health, Matthew Kocher, Scott Fry, and James Usury and Micronutrients, Inc., and Marty Heintz and New Horizon Farms. I am very grateful for their support on these projects.

To Mom, Dad, Hayley, Hadley, Peyton, Holden, Hudson, and Hesson, I would not be who I am without your love, support, and patience. I am forever grateful for this.

Finally, to my wife Kirstin, I will forever be grateful for all the support you have given me throughout these years to complete my program and time here at K-State.

# Dedication

This dissertation is dedicated to my parents Noel and Jacque Williams, and my wife, Kirstin.

# Preface

This dissertation is original work completed by the author, H.E. Williams. All chapters within this dissertation were formatted for publication according to the required standards of the Journal of Animal Science and Translational Animal Science.

# Chapter 1 - Effect of increasing Fe dosage in newborn pigs on suckling and subsequent nursery performance and blood criteria

## 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 a Fe macro-molecule complex that is commercially used as an injectable Fe source for suckling piglets. On the day of processing (d 3 after birth), all piglets were weighed and six barrows and six 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 d 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 d 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 (ADFI), and d 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 less than 150 mg of injectable Fe had decreased values to d 21 and then increased to d 63 while pigs with 150 or 200 mg of injectable Fe had increased values to d 21 then stayed relatively constant to d 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 d 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

**List of abbreviations**:

ADG, average daily gain

ADFI, average daily feed intake

BIC, Bayesian Information Criterion

BW, bodyweight

CP, crude protein

DM, dry matter

EDTA, ethylenediaminetetraacetic acid

ELISA, enzyme-linked immunosorbent assay

$FeSO_4$, ferrous sulfate

G:F, gain-to-feed ratio

Hb, hemoglobin

Hct, hematocrit

Hp, haptoglobin

IFN-γ, interferon-gamma

I.M., intramuscular

LPS, lipopolysaccharide

PHA, phytohemagglutinin

TIBC, total iron binding capacity

TNF-α, tumor necrosis factor-alpha

TPS, trypticase soy agar

# 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. 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 if 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 d 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 to 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 macro-molecule 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 post-weaning 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 blood criteria.

# MATERIALS AND METHODS

## *General*

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 six barrows and six 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., Lenexa, KS) 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 d 3 plus another 100 mg injection on d 11. Each 1 mL of Fe 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. Piglets were weighed at processing (d 3), d 11, and weaning (d 21) to calculate average daily gain (ADG) during lactation. Creep feed was not offered to suckling pigs.

### *Nursery*

Pigs were weaned at approximately 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 tri-bar 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 d 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. Phase 2 and 3 diets were prepared at the Kansas State University O.H. Kruse Feed Technology and Innovation Center located in Manhattan, KS. Phase 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 (FeSO4) 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.

### *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, Kearney, NE) for analysis of dry matter (DM; 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 (EDTA) and whole blood (Monoject, Covidien, Dublin, Republic of Ireland) 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 Fe 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), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and plasma blood kill. Haptoglobin was measured using colorimetric method based on peroxidase activity (Cooke and Arthington, 2013). Tumor necrosis factor-alpha secretion 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 secretion 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., St. Paul, MN) and absorbance measured using a microtech spectrometer (BioTek EON, Winooski, VT). Plasma blood killing was measured by plating a 1:4 dilution of blood plasma:E. coli 51813 on trypticase soy agar (TPS) 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 blood killing.

### *Fecal and Water Analysis*

Fecal samples from 8 sows were collected on d 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., Kearney, NE).

### *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 gender served as random effects in the growth model and crate served as a random effect in the immunological criteria model. Nursery growth data were 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 greater than 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, pre-planned contrasts were utilized to evaluate linear and quadratic effects of Fe dosage from 0 mg 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 mg/kg Fe on d 3, 11, and 21, respectively. Results of water analysis collected from nursery pens averaged 0.03, 0.01, and 0.03 mg/kg Fe on d 0, 14, and 42, respectively. These analyses revealed water contained low levels of Fe as expected because water originated from a municipal water source. Results of fecal

analysis collected from sows during lactation averaged 1,735, 1,610, and 1,140 mg/kg Fe on d 3, 11, and 21, respectively. These analyses revealed high amounts of Fe were present within the feces of sows', but acquisition of Fe and other minerals through ingestion of sow feces has been shown to be minimal (Barros et al., 2019). Analyzed Fe content in the phase 1 nursery diet was higher than formulated values, while phase 2 and 3 analyzed values were slightly lower than expected (Table 2). However, all dietary Fe levels were well above the requirement of nursery pigs (NRC, 2012).

### *Lactation Growth Performance*

From d 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 3). Day 11 BW of piglets increased (quadratic; $P = 0.018$) with increasing injectable Fe up to 50 mg with no further improvement thereafter. From d 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 d 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 d 0 to 10 after weaning, increasing injectable Fe administered post-farrowing improved (linear; $P < 0.05$) ADG, ADFI, and G:F (Table 4). Furthermore, increasing injectable Fe up to 100 mg improved (quadratic; $P = 0.001$) d 10 BW with little improvement thereafter. From d 10 to 24, increasing injectable Fe administered post-farrowing improved (quadratic; $P < 0.05$) ADG and G:F. Increasing injectable Fe up to 150 mg also improved (quadratic; $P = 0.009$)

d 24 BW with no further improvement with increased dosage. From d 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 d 24 to 42, increasing injectable Fe improved (linear; $P < 0.05$) ADG and d 42 ending BW, and marginal significance for improved (linear; $P = 0.071$) ADFI was observed.

Overall (d 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 d 3 prior to the Fe injection (Table 5). For Hb, a significant treatment × day interaction ($P = 0.001$) was observed. The interaction occurred because pigs receiving less than 150 mg of injectable Fe had decreased Hb values to d 21 and then increased to d 63 with the feeding of common diets. Conversely, for pigs receiving the 150 or 200 mg injections, Hb values increased to d 21 and then stayed relatively constant to d 63. Hb values increased (quadratic; $P = 0.001$) on d 11 and 21, and (linear; $P = 0.001$) d 35 with the 0 mg treatment having the lowest Hb values and the 200 mg treatment having the greatest values. There was no evidence of difference ($P > 0.10$) observed for Hb values measured on d 63. On d 21 and 35, the 200 mg + 100 mg treatment led to an increase ($P < 0.05$) in Hb values compared to 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 d 21 and then increasing to d 63 with the feeding of common diets. For pigs receiving 100 mg of injectable Fe, Hct values increased to d 11 and then decreased to d 21. From d 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 d 21 and then stayed relatively constant to d 63. Increasing injectable Fe increased (quadratic; P = 0.001) Hct values on d 11, 21, and (linear; P = 0.001) d 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 d 63. On d 21 and 35, the 200 mg + 100 mg treatment led to an increase (P < 0.05) in Hct values compared to 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 less than 100 mg of injectable Fe staying relatively constant to d 21 and then increasing to d 63 with the feeding of common diets. Meanwhile, for pigs receiving 100 mg of injectable Fe, serum Fe values increased to d 11 and then decreased to d 21. From d 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 d 11 and then decreased to d 21. Serum Fe values for pigs receiving these treatments increased from d 21 to 35 and then stayed relatively constant to d 63 with the feeding of common diets. Increasing injectable Fe increased serum Fe values on d 11 (linear; P = 0.001), d 21 (quadratic; P = 0.002), and d 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 d 63. On d 21, the 200 mg + 100 mg treatment had an increase (P = 0.030) in serum Fe values compared to 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 d 3 to 21 except for the 200 mg treatment staying constant from d 11 to 21. From d 21 to 35, TIBC values decreased for all treatments while pigs receiving 0 or 50 mg of injectable Fe continued to decrease to d 63 with the feeding of common diets. From d 35 to 63, pigs receiving 100 mg of injectable Fe had relatively constant TIBC while pigs receiving 150 mg or 200 mg of injected Fe increased with the feeding of common diets. Increasing injectable Fe decreased (quadratic; P = 0.001) TIBC values on d 11 and (linear; P = 0.001) on d 21 and 35 with the 0 mg treatment having the greatest 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 d 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 d 21 after birth, there was no evidence of difference (P > 0.10) in Hp values with increasing levels of injectable Fe (Table 6). There was no evidence of difference (P = 0.560) between the 200-mg and 200 + 100 mg treatments on d 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 d 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 (d 21), the 200 mg treatment had a greater ($P = 0.029$) plasma blood kill percentage than the 200 + 100 mg treatment.d

## 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 which is the main site of red blood cell production (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 approximately 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). In addition, it is well understood that suckling pigs undergo rapid growth during the first few weeks of life and this rapid growth rate increases their blood volume by approximately 30% (Thorn, 2011). The NRC (2012) estimates that the suckling pig must retain 21 mg of Fe/kg of BW gain to meet growth requirements and maintain adequate levels of Hb and Fe storages. When growth requirements for suckling pigs are not met, anemia can occur. The anemia related to Fe deficiency (defined as a Hb concentration ≤ 9 g/dl) in swine

is characterized as hypochromic microcytic because hemoglobin synthesis is affected by inadequate Fe absorption and storage (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.

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 (Peters and Mahan et al., 2008; Chevalier, 2019). The observations are similar to results from the current study and further support the need for an Fe injection after birth to support lactation and subsequent nursery growth performance.

Previous studies have established the effectiveness of a single intramuscular (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 weeks post-weaning 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. This would suggest administration of Fe injection dosage greater than 200 mg in a single injection at birth or providing supplemental Fe injections at varying

times during lactation is needed to provide a subsequent growth performance benefit at weaning and in the nursery. Although, research on this subject is conflicting. 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 to 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 versus 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 to a single 200 mg injection of Fe at birth. Almond et al. (2017) observed that adminstration of a second injection of 150 mg of Fe approximately 5 to 7 d after birth provided inconsistent responses in subsequent post-weaning growth compared to a single injection of 150 or 200 mg of Fe at 3 to 5 days 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 to 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 to 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 a Hb concentration > 11 g/dL, Fe deficiency as a Hb concentration > 9g/dL but ≤ 11g/dL, and

anemia as a 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 post-weaning. 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 to the pigs receiving 200 mg of Fe. Gentry et al. (1997) suggests that pigs with greater Hb status have improved energy retention compared to 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 post-weaning compared to 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 to 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 post-weaning 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 – 125.0 μmol/L would be considered Fe deficient.

In the present study, pigs administered less than 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 more serum transferrin was transporting Fe throughout the body and an improved blood Fe status. This would support that an

improved Fe status at weaning elicits improved nursery performance. 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., 2016) . Morales et al. (2018) also observed that serum Fe decreased from d 14 to d 21 (weaning) 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). Researchers have observed that Fe deficiency can lead to cell-mediated deficiencies in immunity and that these defects are not apparent based on the level of Fe deficiency. These defects include reduced neutrophil function, impaired intracellular bactericidal activity, decreased T-lymphocyte number and proliferation, impaired natural killer cell activity, and reduced interleukin-2 production (Oppenheimer, 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. The protein also 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. Although, Marro et al. (2007) found in mice that were absent of the gene encoding Hp production and secretion that Hp regulates ferroportin expression and

regulates Fe transfer from duodenal mucosa to plasma. This suggests that swine receiving greater amounts of injectable Fe should have elevated levels of Hp due to greater Hb concentrations, but this was not observed in the current study.

Tumor necrosis factor-alpha and IFN-γ are critical proinflammatory cytokines in the host defense system and 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. The cytokines exhibit antitumor and antiviral activity, stimulates the recruitment of inflammatory cells and forms granulomas that contain infection, promote macrophage activation and mediate antiviral and antibacterial immunity (Pfeffer, 2003). 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 values with increasing Fe injection was observed. Iron deficiency reduces hepcidin transcription which can lead to overproduction of cytokine production of macrophages (Pagani et al., 2011). This could possibly explain why the lower dosage treatments and pigs that were Fe deficient in the current study had greater TNF-α values at weaning compared to pigs that were not Fe deficient. 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. These cytokines have exhibited synergism during inflammation (Bartee and McFadden, 2013), but this was not observed in the current study.

Bacteria that enter the blood stream are eliminated through complement activation on the bacterial surface. If these immune systems are not optimally functioning or bacteria containing mechanisms that allow them to evade these systems, infection can occur (van der Maten et al., 2017). Bactericidal assays are utilized to directly evaluate bacterial killing regardless of intracellular or extracellular location of bacteria (Chabot-Roy et al., 2006). The ability of the immune system of young swine to eliminate bacteria from the bloodstream is of importance because of the relationship between Fe and bacterial growth, specifically E. coli. 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 days 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 after birth had the highest blood killing percentage of E. coli, but the blood killing increased with 150 mg and 200 mg injections of Fe. This could possibly be due to the pigs withheld from Fe not having enough Fe stores for proliferation of E. coli., but the pigs that were not Fe deficient had a stronger immune system to eliminate bacteria from the blood. Furthermore, the pigs receiving an extra 100 mg dose of Fe had lower blood killing percentages compared to 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 d 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. Tumor necrosis factor-alpha and plasma blood killing values were improved with increasing Fe dosage up to 200 mg, but no effect was observed on Hp or IFN-γ values. 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 post-farrowing. 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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**Table 1.1Nursery diet composition (as-fed basis)[1]**

| Ingredient, % | Phase 1 | Phase 2 | Phase 3 |
|---|---|---|---|
| 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 | 19 |
| 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 |
| Calculated Analysis[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 d 0 to 10 (~5.7 to 6.2 kg), Phase 2 diets fed from d 10 to 24 (~6.2 to 11.1 kg), and Phase 3 diets fed from d 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 sulphate; 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. (Kearney, NE) for analysis.

**Table 1.2 Effects of injectable Fe dosage on preweaning pig performance[1]**

| | Fe, mg[2] | | | | | | | Probability, $P <$ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 50 | 100 | 150 | 200 | 200 + 100[3] | SEM | Linear[4] | Quadratic[5] | 200 vs. 200 + 100[6] |
| BW, kg | | | | | | | | | | |
| d 3[7] | 1.7 | 1.7 | 1.7 | 1.8 | 1.7 | 1.8 | 0.05 | 0.793 | 0.943 | 0.556 |
| d 11[8] | 3.3 | 3.6 | 3.5 | 3.6 | 3.5 | 3.5 | 0.01 | 0.012 | 0.018 | 0.702 |
| d 21 | 4.7 | 5.7 | 5.9 | 5.8 | 5.8 | 5.7 | 0.15 | 0.001 | 0.001 | 0.800 |
| ADG, g | | | | | | | | | | |
| d 3 to 11 | 192 | 230 | 228 | 227 | 226 | 218 | 8.2 | 0.002 | 0.002 | 0.409 |
| d 11 to 21 | 154 | 223 | 244 | 229 | 234 | 233 | 8.3 | 0.001 | 0.001 | 0.772 |
| d 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., Lenexa, KS) 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 mg to 200 mg dosage.

[5]Quadratic comparison of 0 mg to 200 mg dosage.

[6]Pairwise comparison between mean of 200 mg and 200 + 100 mg treatments.

[7]Represents 3 d after birth.

[8]Represents 11 d after birth.

**Table 1.3 Effects of injectable Fe dosage on nursery pig performance[1]**

| | Fe, mg[2] | | | | | | | Probability, *P* < | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | 0 | 50 | 100 | 150 | 200 | 200 + 100[3] | SEM | Linear[4] | Quadratic[5] | 200 vs. 200 +100[6] |
| BW, kg | | | | | | | | | | |
| d 0 | 4.9 | 5.7 | 5.9 | 5.8 | 5.8 | 5.8 | 0.08 | 0.001 | 0.001 | 0.997 |
| d 10 | 5.2 | 6.0 | 6.3 | 6.4 | 6.4 | 6.6 | 0.27 | 0.001 | 0.001 | 0.339 |
| d 24 | 9.3 | 10.6 | 11.2 | 11.7 | 11.6 | 12.1 | 0.27 | 0.001 | 0.009 | 0.277 |
| d 42 | 19.4 | 21.1 | 21.6 | 22.7 | 22.9 | 22.6 | 0.53 | 0.001 | 0.209 | 0.730 |
| d 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 |
| d 10 to 24 | | | | | | | | | | |
| ADG, g | 283 | 327 | 354 | 378 | 365 | 376 | 15.6 | 0.001 | 0.036 | 0.563 |
| ADFI, g | 510 | 507 | 505 | 543 | 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 |
| d 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 | 0.588 | 0.658 | 0.661 | 0.642 | 0.658 | 0.0263 | 0.001 | 0.017 | 0.636 |
| d 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 | 0.157 | 0.323 | 0.787 |
| d 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 | 630 | 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., Lenexa, KS) 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 mg to 200 mg dosage.

[5]Quadratic comparison of 0 mg to 200 mg dosage.

[6]Pairwise comparison between mean of 200 mg and 200 + 100 mg treatments.

**Table 1.4 Effects of injectable Fe dosage on suckling and nursery pig hematological criteria[1]**

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

[5]Quadratic comparison of 0 mg to 200 mg dosage.

[6]Pairwise comparison between mean of 200 mg 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.

**Table 1.5 Effects of injectable Fe dosage on immune criteria at weaning (21-d post-farrowing)[1]**

| | Fe, mg[2] | | | | | | Probability, *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 d 21 after farrowing. Immune function criteria were measured from 1 barrow per treatment per sow.

[2]Fe (Gleptoforte, Ceva Animal Health, LLC., Lenexa, KS) 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 mg to 200 mg dosage.

[5]Quadratic comparison of 0 mg to 200 mg dosage.

[6]Pairwise comparison between mean of 200 mg and 200 + 100 mg treatments.

# Chapter 2 - Effects of iron injection timing on suckling and subsequent nursery and growing-finishing performance and hematological criteria


## ABSTRACT

Two experiments were conducted to evaluate the effects of Fe injection timing after birth on suckling and subsequent nursery and growing-finishing pig performance. The injectable Fe source used in both experiments was GleptoForte (Ceva Animal Health, LLC., Lenexa, KS). GleptoForte contains gleptoferron which is a Fe macro-molecule complex. In Exp. 1, a total of 324 newborn pigs (DNA 241 × 600, initially 1.6 kg body weight [BW]) within 27 litters were used. Two days after birth, all piglets were weighed, and six barrows and six gilts were allotted to 1 of 6 treatments consisting of no Fe injection or 200 mg of injectable Fe provided in a single injection on d 2, 4, 6, 8, or 10 of age. Pigs were weaned (~21 d of age) and allotted to nursery pens based on previous Fe treatment. In Exp. 2, a total of 1,892 newborn pigs (PIC 359 × C40; initially 1.5 kg BW) within 172 litters were used. One day after birth, piglets were weighed, and 11 pigs within each litter were allotted to 1 of 6 treatments consisting of no Fe injection or 200 mg of injectable Fe provided on d 1, 3, 5, or 7 of age, or 200 mg on d 1 plus 200 mg on d 12 of age. Pigs were weaned (19 d of age) and placed in a commercial wean-to-finish facility in a total of 15 pens with equal representation of treatments in each pen. In both experiments, not providing an Fe injection after birth decreased ($P > 0.05$) preweaning ADG and weaning weight compared to all other treatments. In Exp. 1, increasing the age that piglets received an Fe injection until 4 or 6 d after birth provided marginal evidence for an improvement (quadratic; $P = 0.070$) in preweaning average daily gain (ADG). For the nursery period, increasing the age that piglets received an Fe injection improved (quadratic; $P = 0.013$) d 80 BW, but there was no evidence of a difference ($P > 0.10$) in d 173 BW at the end of the grow-finish period. In Exp. 2, increasing the age that piglets received a 200 mg Fe injection showed no evidence of difference ($P>0.10$) for subsequent nursery and growing-finishing ADG. In both experiments, hemoglobin and hematocrit values were decreased (linear; $P < 0.05$) at weaning with increasing age when pigs received an Fe injection. These experiments suggest that providing a 200 mg Fe injection

within 7 d after farrowing is sufficient for optimizing preweaning and subsequent growth performance.

**Key words:** Fe, gleptoferron, growth performance, timing

**List of abbreviations:**

ADG, average daily gain

ADFI, average daily feed intake

BW, bodyweight

EDTA, ethylenediaminetetraacetic acid

FeSO4, iron sulfate

G:F, gain-to-feed

Hb, hemoglobin

Hct, hematocrit

IM, intramuscular

TIBC, total iron binding capacity

## INTRODUCTION

Iron is an indispensable micromineral due to its involvement in many biological functions such as oxygen binding and transport, oxygen metabolism, and cell proliferation and differentiation (Pantopoulos et al., 2012). Iron deficiency and anemia develop prior to weaning because of low Fe storages at birth, rapid growth rate, and low sow colostrum and milk Fe content (Kegley et al., 2002; Hurley, 2015). Therefore, efficacy of supplemental injectable Fe after birth on suckling and subsequent nursery pig performance is well established (Peters and Mahan, 2008; Chevalier, 2019; Williams et al., 2019). A single 200 mg intramuscular (IM) injection of Fe is commonly used in the swine industry to prevent Fe deficiency.

The adminstration of a single 200 mg Fe dose is commonly practiced within the first week post-farrowing. However, limited research exists on the specific day of age when the Fe injection is administered to optimize performance and blood Fe status. Egeli and Framstad

(1999) determined that administering a 180 mg injection of Fe dextran to suckling pigs 1, 3, or 4 days after birth showed no evidence of difference in Hb values 14 or 21 d after birth. Furthermore, Kernkamp et al. (1962) observed that increasing the age at which a 150 mg Fe dextran injection was administered from 7 to 14 or 21 d of age decreased hemoglobin (Hb) and hematocrit (Hct) values at 21 and 28 d after birth but showed no evidence of a difference in BW up to 56 d of age.

Gleptoferron is a commercially available injectable Fe source. The optimal dose of Fe from gleptoferron in pigs was previously determined to be 200 mg to optimize growth and blood Fe status (Williams et al., 2019). In that study, all pigs were administered the Fe injection on d 3 after farrowing. However, we are unaware of any studies evaluating the timing of gleptoferron adminstration after birth. Therefore, the objective of these studies were to evaluate the effects of increasing the age when newborn pigs receive a 200 mg Fe injection provided from gleptoferron on preweaning and subsequent nursery and growing-finishing performance and hematological criteria.

## MATERIALS AND METHODS

### *General*

The Kansas State University Institutional Animal Care and Use Committee approved the protocols for these experiments. Experiment 1 was conducted at the Kansas State University Swine Teaching and Research Center located in Manhattan, KS. Farrowing stalls were equipped with an individual water nipple and an electronic feeding system (Gestal Solo Feeders, Jyga Technologies, Quebec City, Quebec) to provide sows with ad libitum access to feed and water. Each nursery pen (1.52 × 1.52 m) had metal tri-bar flooring, one 4-hole self-feeder, and a nipple waterer to provide ad libitum access to feed and water. Each growing-finishing pen (1.52 × 3.05

m) was equipped with a single-hole stainless steel feeder and 2 nipple waterers to provide ad libitum access to feed and water. Experiment 2 was conducted at a commercial sow facility in northwest Texas and wean-to-finish facility in central Iowa. Farrowing crates were equipped with an individual water nipple and feeder to provide sows with ad libitum access to water and feed. The wean-to-finish facility was naturally ventilated and double-curtained-sided. Each wean-to-finish pen (9.80 × 7.60 m) was equipped with a 5-hole stainless steel wet-dry feeder and 2 nipple waterers to provide ad libitum access to feed and water.

### *Animals*

In Exp. 1, a total of 324 newborn pigs (DNA 241 × 600, initially 1.6 ± 0.04 kg bodyweight [BW]) were used in a 173-d study. A total of 27 litters were used with the number of pigs per sow equalized on each day of farrowing. Two days after birth, all piglets were weighed, and six barrows and six gilts were allotted in a completely randomized design to 1 of 6 treatments such that there was 1 barrow and 1 gilt per treatment for each sow. Thus, there were 54 replications per treatment. The six treatments consisted of a negative control receiving no Fe injection or 200 mg of injectable Fe (GleptoForte, Ceva Animal Health, LLC., Lenexa, KS) provided in a single injection on d 2, 4, 6, 8, or 10 after birth. Piglets were weighed on d 2, 12, and weaning (d 21) after birth to calculate preweaning average daily gain (ADG). Creep feed was not offered to suckling pigs. Pigs were weaned at approximately 21 d of age and allotted to pens based on previous Fe treatment with BW balanced across all pens within a treatment with 5 or 6 pigs per pen and 10 pens per treatment. Pigs and feeders were weighed on d 28, 35, 42, 48, 55, 62, and 80 after birth to determine ADG, average daily feed intake (ADFI), and gain-to-feed (G:F). On d 80 after birth, pigs receiving no Fe injection or 200 mg of injectable Fe from GleptoForte provided in a single injection on d 2, 4, or 10 after birth were allotted to pens based

on previous Fe treatment and gender with BW balanced across all pens within a treatment with 5 or 6 pigs per pen and 9 or 10 pens per treatment. Treatments followed into the grow-finish period were pre-determined due to limited number of pens in the facility. Pigs and feeders were weighed on d 108, 138, and 173 after birth to determine ADG, ADFI, and G:F.

In Exp. 2, a total of 1,892 newborn pigs (PIC 359 × C40; initially 1.5 ± 0.02 kg BW) within 172 litters were used in a 168 d study. One d after birth, all piglets were individually weighed, and 11 piglets within each litter were allotted to 1 of 6 treatments in a completely randomized design. One pig per litter received no Fe injection and 2 pigs per litter were used on all other treatments. Thus, there were 172 replications for the no Fe injection treatment and 344 replications for all other treatments. Treatments consisted of pigs receiving no Fe injection or 200 mg of injectable Fe (GleptoForte, Ceva Animal Health, Lenexa, KS) provided on d 1, 3, 5, or 7 of age, or 200 mg on d 1 plus 200 mg on d 12 of age. Piglets were individually weighed on d 1 and weaning (d 19) to determine preweaning ADG. Creep feed was not offered to suckling pigs. At weaning, pigs were placed in a commercial wean-to-finish facility in a total of 15 pens with equal representation of treatments in each pen. Pigs were individually weighed on d 72 and 168 after birth to determine subsequent nursery and growing-finishing ADG.

### *Diet Preparation*

In both experiments, common diets were fed in all nursery and growing-finishing phases. The phase 1 nursery diets were fed in pellet form. Phase 2 and 3 nursery diets and all growing-finishing diets (3 total) were fed in meal form. All nursery diets were supplemented with 110 mg/kg Fe from iron sulfate (FeSO4) provided by the trace mineral premix. Inclusion rate of the trace mineral premix in growing-finishing diets was reduced in a step-wise manner and diets contained 92, 73, and 55 mg/kg supplemental Fe from FeSO4. All diets were formulated

according to the Nutrient Requirements of Swine (NRC, 2012) to be at or above the pig's daily nutrient requirements to not limit growth performance.

### *Chemical Analysis*

For both experiments, complete diet samples for each nursery dietary phase were taken directly from feeders and stored at -20°C. Diet samples were pooled, subsampled, and sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for analysis of Fe (AOAC 999.11, 2012).

### *Blood analysis*

In Exp. 1, blood samples were collected via jugular venipuncture in 5-mL ethylenediaminetetraacetic acid (EDTA) and whole blood (Monoject, Covidien, Dublin, Republic of Ireland) tubes using 22-gauge, 2.54 cm needles from one barrow per treatment per litter on d 2, 12, and 21 after farrowing as well as d 35 after birth. Hematological criteria measured included Hb and Hct using an ADVIA 2021i Hematology System (Siemens Healthcare Diagnostics, Tarrytown, NY) and serum Fe and total Fe 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 located in Manhattan, KS.

In Exp. 2, whole blood samples were collected via jugular venipuncture in 5-mL lithium heparin (Becton, Dickinson and Company, Franklin Lakes, NJ) vacutainer tubes using 22-gauge, 2.54 cm needles from 30 barrows per treatment at weaning (d 20). Whole blood samples were immediately analyzed for Hb and Hct on a handheld iSTAT portable clinical analyzer (iStat Alinity, Abbott Point of Care Inc.; Princeton, NJ).

### *Statistical Analysis*

In Exp. 1 and 2, growth data of suckling piglets were analyzed as a completely randomized design with individual pig as the experimental unit and sow × gender serving as the random effect. In Exp. 1, nursery and growing-finishing growth data were analyzed as a randomized complete block design with pen as the experimental unit. Block was included in the model as a random effect and accounted for location within the room at the time of allotment. In Exp. 2, nursery and growing-finishing growth and lifetime growth data were analyzed with individual pig as the experimental unit. Sow × gender and pen served as random effects in the model.In Exp. 2, heterogenous variance was accounted for where appropriate. The BIC was used to determine best fit, with a lower number indicating an improved fit. A decrease in BIC greater than 2 among models for a hematological criterion was considered a significant improvement in fit (Goncalves et al., 2016). All growth and hematological criteria were analyzed assuming a normal distribution and mortality data was analyzed using a binomial distribution.

In both experiments, pre-planned contrasts were utilized to evaluate linear and quadratic effects of Fe injection timepoint after birth and a pairwise comparison of the negative control vs. all other treatments. In Exp. 2, a pairwise comparison was utilized to evaluate pigs receiving a 200 mg injection on d 1 after birth vs. pigs receiving a 200 mg injection on d 1 after birth plus 200 mg on d 12 after birth. Differences between treatments were determined by using least squares means. Data were analyzed using the GLIMMIX procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $P > 0.05$ and $P \leq 0.10$.

# RESULTS

## *Chemical Analysis*

Iron analysis of the nursery diets indicated that diets either met or exceeded the pig's Fe requirement estimate (NRC, 2012; Table 1). The analyzed Fe content averaged 100 mg/kg across all nursery diets phases in Exp. 1 and 107 mg/kg in Exp. 2.

## *Experiment 1*

From d 2 to 21 (weaning) of age, marginal evidence for an improvement (quadratic; P = 0.070) in preweaning ADG was observed when increasing the age piglets received a 200 mg Fe injection to 4 or 6 d after birth with a decrease in performance observed when delaying Fe injection until d 8 or 10. Increasing the age piglets received a 200 mg Fe injection to 4 or 6 d after birth provided marginal evidence (quadratic; P = 0.072) for an improvement in d 21 BW. Not providing a Fe injection resulted in decreased (P = 0.0001) overall preweaning ADG and d 21 BW compared to all other treatments.

During the nursery period (d 21 to 80 of age), there was no evidence of a difference (P > 0.10) in growth performance by increasing the age when piglets received a 200 mg Fe injection. Increasing the age piglets received a 200 mg Fe injection to 4 or 6 d after birth increased (quadratic; P = 0.012) d 80 ending BW. The absence of a Fe injection after birth decreased (P < 0.05) nursery ADG, ADFI, and d 80 ending BW compared to all other treatments.

During the finishing period (d 80 to 173 of age), there was no evidence of a difference (P > 0.10) in growth performance amongst the pigs receiving an Fe injection after birth. The absence of a Fe injection after birth increased (P = 0.048) ADG, but there was no evidence of a difference (P > 0.10) in ADFI or G:F amongst the treatments. There was no evidence of a difference (P > 0.10) amongst the treatments for d 173 ending BW.

For Hb and Hct, a significant treatment × day interaction (P = 0.001) was observed (Table 3). The interaction occurred because pigs receiving a 200 mg Fe injection on d 2, 4, 6, or 8 after birth had increasing Hb and Hct values to d 12 of age, while pigs not receiving a 200 mg Fe injection or pigs receiving a 200 mg Fe injection on d 10 after birth had decreasing Hb values to d 12 of age. All pigs receiving a 200 mg Fe injection had increased Hb and Hct values from d 12 to 21 of age and then slightly decreased to d 35 of age, while pigs not receiving an Fe injection had decreased values from d 12 to 21 of age and then increasing Hb and Hct values to d 35 of age. As expected, there was no evidence of difference (P > 0.10) observed for any hematological criteria measured on d 2 of age prior to the Fe injection. On d 12 of age, administering a 200 mg Fe injection up to d 4 after birth increased (quadratic; P = 0.001) Hb and Hct values with a decrease in values observed with later injections. On d 21 of age, administering a 200 mg Fe injection after d 6 of birth decreased (linear; P = 0.047) Hb values. On d 21 of age, increasing the age at which pigs received an Fe injection provided marginal evidence (linear; P = 0.094) for a decrease in Hct. On d 35 of age, there was no evidence of difference (P > 0.10) in Hb and Hct values amongst the treatments. The absence of a Fe injection after birth resulted in decreased (P = 0.001) Hb and values on d 12, 21, and 35 of age compared to all other treatments.

For serum Fe, a significant treatment × day interaction (P = 0.01) was observed. The interaction was a result of serum Fe values in pigs receiving a 200 mg Fe injection after birth increasing from d 2 to 12, decreasing from d 12 to 21, and then increasing from d 21 to 35 of age while pigs not receiving an Fe injection had decreasing serum Fe values to d 21 of age, then increasing values from d 21 to 35 of age. On d 12 of age, serum Fe values increased (quadratic; P = 0.001) when the 200 mg of Fe injection was administered up to d 10 with a larger increase in values from d 8 to d 10 when Fe was administered. On d 21 and 35 of age, there was no evidence

of difference (P > 0.10) in serum Fe values amongst the pigs receiving a 200 mg Fe injection. The absence of a Fe injection decreased (P = 0.001) serum Fe values on d 12, 21, and 35 of age compared to all other treatments.

A significant treatment × day interaction (P = 0.001) was observed for TIBC values. This interaction occurred because pigs receiving a 200 mg Fe injection on d 2, 4, or 6 after birth had increased TIBC values up to d 21 of age then decreased to d 35 of age while pigs receiving a 200 mg Fe injection 8 or 10 d after birth had increasing TIBC values up to d 12 of age then decreasing values from d 12 to 35 of age. Pigs not receiving an Fe injection after birth had increasing TIBC values up to d 21 of age and then decreased to d 35 of age. On d 12 of age, TIBC values increased (quadratic; P = 0.001) when the 200 mg Fe injection was administered to d 10 after birth with a larger increase in values observed when Fe was administered from d 6 to d 10. On d 21 and 35 of age, there was no evidence of difference (P > 0.10) observed for TIBC values in pigs receiving a 200 mg Fe injection. The absence of a Fe injection decreased (P = 0.001) TIBC values on d 12, 21, and 35 of age compared to all other treatments.

### *Experiment 2*

From d 1 to 19 of age, marginal significance for a decrease (linear; P = 0.080) in preweaning ADG was observed with increasing the age at which pigs received a 200-mg Fe injection (Table 4). However, there was no evidence for a difference (P > 0.10) in d 19 BW with increasing age at which pigs received a 200-mg Fe injection. The absence of an Fe injection decreased (P = 0.0001) preweaning ADG and d 19 BW compared to pigs receiving an Fe injection. Providing a 200-mg Fe injection on d 1 plus d 12 of age showed no evidence of a difference (P > 0.10) in preweaning ADG or d 19 BW compared to pigs receiving a 200-mg Fe injection on d 1 only.

From d 19 (weaning) to 72 of age, increasing the age at which pigs received a 200-mg Fe injection after birth provided no evidence for a difference ($P > 0.10$) in subsequent nursery ADG. However, marginal significance for a decrease ($P = 0.060$) in d 72 ending BW was observed with increasing age post-farrowing when pigs received a 200-mg Fe injection. The absence of an Fe injection post-farrowing decreased ($P = 0.0001$) subsequent nursery ADG and d 72 ending BW. Providing a 200-mg Fe injection on d 1 plus d 12 of age decreased ($P < 0.05$) subsequent nursery ADG and d 72 ending BW compared to pigs receiving a 200-mg Fe injection on d 1 of age only.

From d 72 to 168 of age, increasing the age at which pigs received a 200-mg Fe injection provided no evidence for a difference ($P > 0.10$) in subsequent growing-finishing ADG or d 168 ending BW. The absence of an Fe injection post-farrowing decreased ($P < 0.05$) subsequent growing-finishing ADG and d 168 ending BW. Providing a 200-mg Fe injection on d 1 plus d 12 of age showed no evidence of a difference ($P > 0.10$) in subsequent growing-finishing ADG and d 168 ending BW compared to pigs receiving a 200-mg Fe injection on d 1 of age only.

Overall from d 1 to 168 of age, increasing the age at which pigs received a 200-mg Fe injection provided no evidence for a difference ($P > 0.10$) in overall ADG. The absence of an Fe injection post-farrowing decreased ($P = 0.0001$) overall ADG. Providing a 200-mg Fe injection on d 1 plus d 12 of age showed no evidence of a difference ($P > 0.10$) in overall ADG compared to pigs receiving a 200-mg Fe injection on d 1 of age only.

There was no evidence ($P > 0.10$) that Fe injection treatments influenced preweaning or wean-to-finish mortality. The absence of an Fe injection post-farrowing provided no evidence for a difference ($P > 0.10$) in preweaning or wean-to-finish mortality.

For hematological criteria, increasing the age at which pigs received an Fe injection decreased ($P < 0.05$) Hb and Hct values at d 19 of age (Table 5). The absence of an Fe injection

post-farrowing decreased (P = 0.0001) Hb and Hct values at d 19 of age compared to pigs receiving an Fe injection after birth. Providing a 200-mg Fe injection on d 1 plus d 12 of age increased (P = 0.0001) Hb and Hct values at weaning compared to pigs receiving a 200-mg Fe injection on d 1 only.

## DISCUSSION

Iron is an essential micronutrient that is vital for maintaining homeostasis in mammals as it has involvement in numerous cellular processes such as such as electron transport, DNA synthesis, energy production and mitochondria function (Aisen et al., 2001). Because of low Fe storages at birth and milk Fe being inadequate to meet growth and maintenance needs, suckling pigs are at a high risk for developing Fe deficiency anemia. This is due to decreases in Hb synthesis because of inadequate Fe absorption and storage (Dallman, 1986). To prevent anemia and support growth, a single IM injection of 200 mg of Fe is commonly used in the swine industry. The detrimental effects of the absence of an Fe injection on preweaning and subsequent nursery growth performance have been shown (Chevalier, 2019; Williams et al., 2019). The studies herein would agree as preweaning and subsequent nursery growth performance of pigs not receiving an Fe injection after birth was decreased in both experiments.

Research on the effects the absence of an Fe injection after birth on subsequent growing-finishing performance has not previously been researched. In Exp. 1, pigs that did not receive an Fe injection after birth had similar ending BW and improved growth performance in the finishing portion of the study compared to those pigs that received an Fe injection post-farrowing. However, in Exp. 2, pigs that did not receive an Fe injection after birth had ending BW that were approximately 7 kg lighter than that of pigs receiving an Fe injection after birth. The reason for

the discrepancies in finisher performance of pigs not receiving an Fe injection is unknown and warrants further investigation.

Limited research exists to define the specific day of age when the Fe injection is administered to optimize performance and blood Fe status. Kernkamp et al. (1962) observed that, in pigs weaned at 28 d of age, administering 150 mg of Fe dextran on d 7, 14, or 21 after birth resulted in a decrease in Hb and Hct values as day of injection increased; although they observed no evidence of difference in growth performance up to 56 d after birth. Egeli and Framstad (1999) conducted 2 separate experiments to evaluate the effects of administering a single 180 mg Fe injection 1, 3, or 4 d after birth on blood criteria in newborn pigs. Observations from the 1st experiment showed that providing the Fe injection either 1 or 3 d after birth resulted in evidence of a difference in Hb values at 14 or 21 d of age. Observations from the 2nd experiment showed no evidence of difference in Hb values at 14 d of age. The studies herein agree with Kernkamp et al. (1962) in that increasing the age at which pigs received an Fe injection decreased Hb and Hct values at weaning; however, our studies did show that growth performance was influenced when the age at which pigs receive an Fe injection was increased, which is opposite of what Kernkamp et al. (1962) reported. The inconsistencies could be due to weaning age as the pigs in the studies herein were weaned at ~21 d of age while the pigs in Kernkamp et al. (1962) were weaned at 28 d of age. Inconsistencies between Hb values reported in the Egeli and Framstad (1999) studies and the studies herein are also noted which may be reflective of the timing at which measurements were taken as Egeli and Framstad (1999) only took blood values up to 14 d after birth in the second experiment while Hb values were measured at weaning in both experiments herein. Differences in the analytical procedures used to evaluate Hb and Hct between the studies could also explain these inconsistences. This can be observed in the studies herein as Hct values

were similar between the 2 experiments, but Hb values were comparatively higher in Exp. 1 compared to Exp. 2. Furthermore, the studies herein show differing effects of age when suckling piglets receive a 200 mg injection of Fe from gleptoferron on preweaning and subsequent growth performance. Observations from Exp. 1 show that administering a 200-mg Fe injection from gleptoferron on d 4 or 6 showed marginal significance for improvements in preweaning growth performance and ending nursery BW. Observations from Exp. 2 show that increasing the age at which pigs receive a 200-mg of Fe from gleptoferron marginally decreased preweaning growth performance and show no evidence of differences in subsequent nursery or grow-finish growth performance. Additional research is warranted to better understand these differences in post-wean growth performance response related to day of Fe injection.

Recent research has shown that a single IM injection of 200-mg of Fe from gleptoferron is effective in supporting preweaning and nursery growth requirements (Morales et al., 2018; Williams et al., 2019). Although, some researchers suggest that a single IM injection of 200 mg of Fe is insufficient in supporting pre- and post-weaning growth (Van Gorp et al., 2012; Bhattarai and Nielsen, 2015). Perri et al. (2016) observed that pigs exhibiting low Fe status at weaning were 0.82 kg lighter 3 weeks post-weaning than pigs with normal Fe status. This research would suggest improving blood Fe status at weaning through an additional injection of Fe during the preweaning period would improve subsequent nursery growth performance, but research is conflicting. Chevalier (2019) 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 improved subsequent nursery growth performance compared to pigs receiving a single injection of 150 mg of Fe 1 d after birth. However, Almond et al. (2017) observed that administration of a second injection of 150 mg of Fe approximately 5 to 7 d before weaning provided inconsistent

responses in subsequent post-weaning growth compared to a single injection of 150 or 200 mg of Fe at 3 to 5 days of age. Williams et al. (2019) also observed no evidence of difference in weaning weight or subsequent nursery growth performance when pigs were provided a single 200 mg Fe injection on d 3 after birth compared to pigs receiving a 200 mg of Fe on d 3 after birth and an additional 100 mg 10 d after birth. The results of experiment 2 would agree with observations of Almond et al. (2017) and Williams et al. (2019) as providing an additional 200 mg injection of Fe 12 d after birth showed no evidence of a difference in preweaning growth performance compared to a single injection on d 1. One possibility for the discrepancies is the varying timepoints and dosages in which the additional Fe is administered during lactation and warrants further investigation. Furthermore, the study herein observed a decrease in subsequent nursery ADG and ending BW when pigs were administered an additional 200 mg of Fe 12 d after birth. Langie et al. (2010) observed that oxidative stress was induced in suckling piglets when administered an IM injection of 200 mg of Fe 3 d after birth. The researchers speculate the potential oxidative stress induced by the additional 200 mg Fe injection potentially could negatively influence preweaning growth performance. Interestingly, administration of an additional Fe injection 12 d after birth decreased nursery growth performance but showed no evidence for an effect on preweaning growth performance. The potential effects of oxidative stress with an additional 200 mg injection of Fe after birth on subsequent nursery growth performance should be further explored.

Research has suggested that Hb and Hct, normally used as indicators to determine Fe deficiency, may underestimate the Fe requirement for blood Fe status. This is because Hb and Hct may not accurately indicate early Fe deficiency because erythrocytes have a slow turnover rate and are measures of erythropoietic activity in mature erythrocytes (Cook, 2005). Therefore,

some research suggests that serum Fe and TIBC may be more suitable indicators to determine Fe deficiency as they are earlier indicators of erythropoietic activity in piglets (Bhattarai and Nielsen, 2015). Limited reference values are available for serum Fe and TIBC in swine that would indicate Fe deficiency. Perri et al. (2017) suggest 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. To our knowledge, Exp. 1 is the first to report the effects of age when suckling piglets receive a 200 mg Fe injection from gleptoferron after birth on serum Fe and TIBC values. The results suggest that pigs provided a single 200 mg Fe injection from gleptoferron from d 2 to 10 after birth had increased serum Fe values from d 2 after birth until weaning. These results would agree with Pollmann et al. (1983) that a single injection of 200 mg of Fe from gleptoferron after birth is sufficient to maintain serum Fe values above Fe deficiency levels at weaning. The study also suggests that pigs not receiving an Fe injection after birth would be considered Fe deficient at weaning which could possibly further explain the reductions in preweaning and subsequent nursery growth performance. Morales et al. (2018) also observed that serum Fe decreased from d 14 to 21 (weaning) in pigs injected with 200 mg of Fe after birth from either gleptoferron or Fe dextran, similar to the study herein. Furthermore, results from Exp. 1 show decreased TIBC values at weaning in pigs receiving a 200 mg Fe injection after birth compared to pigs not administered an Fe injection after birth. This results would agree with prior research and indicate more serum transferrin was transporting Fe throughout the body and an improved blood Fe status (Pollmann et al., 1983; Sperling et al., 2018).

In summary, these studies have provided evidence that administering 200-mg of Fe from gleptoferron within 7 d after birth optimizes preweaning and subsequent nursery and grow-finishing growth performance. Administering a Fe injection after birth increased preweaning and

subsequent nursery growth performance and blood Fe status at weaning regardless of timing compared to those that did not receive an Fe injection. Providing an additional 200 mg of Fe 12 d after birth increases Hb and Hct values but did not improve preweaning or subsequent growth performance.

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**Table 2.1 Chemical analysis of nursery diets (as-fed basis), Exp. 1 and 2[1]**

| | Exp. 1 | | | Exp. 2 | | |
|---|---|---|---|---|---|---|
| Item | Phase 1 | Phase 2 | Phase 3 | Phase 1 | Phase 2 | Phase 3 |
| Fe, mg/kg | 101 | 112 | 88 | 113 | 110 | 98 |

[1]Complete diet samples for each dietary phase were taken directly from feeders and stored at -20°C. Diet samples were pooled, subsampled and sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for analysis of Fe.

**Table 2.2 Effects of injectable Fe timing on preweaning and subsequent nursery and growing-finishing growth performance, Exp.1[1]**

| | Fe injection day[2] | | | | | | | Probability, *P* = | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[3] | 0[4] | 2 | 4 | 6 | 8 | 10 | SEM | Linear[5] | Quadratic[5] | 0 vs. Others[6] |
| BW, kg | | | | | | | | | | |
| d 2 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 1.6 | 0.04 | 0.697 | 0.376 | 0.822 |
| d 21 | 5.4 | 5.9 | 6.2 | 6.2 | 5.8 | 5.9 | 0.16 | 0.253 | 0.072 | 0.0001 |
| d 80 | 32.9 | 34.5 | 36.8 | 37.1 | 36.3 | 35.8 | 0.74 | 0.341 | 0.012 | 0.0001 |
| d 173 | 132.1 | 131.5 | 132.2 | --- | --- | 133.6 | 1.39 | 0.260 | 0.820 | 0.820 |
| d 2 to 21 | | | | | | | | | | |
| ADG, g | 202 | 230 | 247 | 245 | 223 | 229 | 7.5 | 0.178 | 0.070 | <0.0001 |
| d 21 to 80 | | | | | | | | | | |
| ADG, g | 456 | 480 | 518 | 501 | 512 | 503 | 13.6 | 0.346 | 0.185 | 0.003 |
| ADFI, g | 742 | 775 | 830 | 813 | 806 | 808 | 19.3 | 0.499 | 0.193 | 0.004 |
| G:F | 0.616 | 0.618 | 0.624 | 0.616 | 0.636 | 0.621 | 0.8635 | 0.463 | 0.628 | 0.366 |
| d 80 to 173[7] | | | | | | | | | | |
| ADG, g | 1,062 | 1,044 | 1,025 | --- | --- | 1,034 | 11.8 | 0.55 | 0.310 | 0.048 |
| ADFI, g | 3,026 | 2,968 | 2,951 | --- | --- | 2,956 | 51.1 | 0.866 | 0.851 | 0.252 |
| G:F | 0.351 | 0.352 | 0.348 | --- | --- | 0.35 | 0.5183 | 0.769 | 0.586 | 0.853 |

[1]A total of 324 suckling pigs (DNA 241 × 600) were used in a 173-d experiment with 12 pigs per sow and 2 replications of treatment within sow. Pigs were weaned at 21 d after farrowing and placed in a research nursery facility with 5 or 6 pigs per pen based on previous Fe treatment and 10 pens per treatment. On d 80 after birth, pigs were placed in a research growing-finishing research facility with 5 or 6 pigs per pen based on previous Fe treatment and 9 or 10 pens per treatment. Treatments evaluated in the growing-finishing phase included pigs receiving no Fe injection or 200-mg of injectable Fe from GleptoForte (Ceva Animal Health, LLC., Lenexa, KS) on d 2, 4, or 10 after birth due to limited number of pens. Common diets were fed throughout the nursery and growing-finishing phases and contained 110 mg/kg added Fe from $FeSO_4$ provided from the trace mineral premix.

[2]200 mg of Fe (GleptoForte, Ceva Animal Health, LLC., Lenexa, KS) administered on d 2, 4, 6, 8, and 10 after farrowing.

[3]BW = bodyweight. ADG = average daily gain. ADFI = average daily feed intake. G:F = gain-to-feed ratio. Each timepoint represents days after farrowing. Days 2 and 21 represent timepoints in farrowing. Days 80 and 173 represent timepoints in the nursery and growing-finishing phases, respectively.

[4]Negative control with pigs receiving no Fe injection.

[5]Comparison of d 2 to d 10 injection treatments.

[6]Comparison between mean of pigs receiving no Fe injection and mean of all other pigs.

[7]Pigs receiving Fe injection treatments on d 6 and d 8 after birth were not evaluated in the growing-finishing period due to limited number of pens able to be utilized.

**Table 2.3 Effects of injectable Fe timing on suckling and nursery pig hematological criteria, Exp. 1[1]**

| | | Fe injection day[2] | | | | | | Probability, *P* < | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[3] | 0[4] | 2 | 4 | 6 | 8 | 10 | SEM | Linear[5] | Quadratic[5] | 0 vs. Others |
| Hb, g/dL[6] | | | | | | | | | | |
| d 2[7] | 8.3 | 8.2 | 8.4 | 8.2 | 8.4 | 8.1 | 0.21 | 0.843 | 0.337 | 0.675 |
| d 12[8] | 5.6 | 10.4 | 11.0 | 10.5 | 9.0 | 7.0 | 0.22 | 0.001 | 0.001 | 0.001 |
| d 21 | 4.1 | 11.4 | 11.5 | 11.4 | 11.2 | 10.9 | 0.21 | 0.047 | 0.187 | 0.001 |
| d 35 | 7.2 | 10.4 | 10.6 | 10.9 | 10.1 | 10.5 | 0.22 | 0.583 | 0.313 | 0.001 |
| Hct, %[6] | | | | | | | | | | |
| d 2 | 28.7 | 28.2 | 29.2 | 28.8 | 28.9 | 27.9 | 0.75 | 0.709 | 0.168 | 0.859 |
| d 12 | 19.6 | 35.4 | 37.0 | 36.5 | 31.2 | 23.5 | 0.69 | 0.001 | 0.001 | 0.001 |
| d 21 | 14.1 | 39.0 | 38.9 | 38.9 | 38.4 | 37.4 | 0.71 | 0.094 | 0.376 | 0.001 |
| d 35 | 26.9 | 35.1 | 35.9 | 37.2 | 34.5 | 35.4 | 0.73 | 0.638 | 0.126 | 0.001 |
| Serum Fe, μg/dL[6] | | | | | | | | | | |
| d 2 | 45 | 47 | 44 | 40 | 51 | 43 | 9.4 | 0.958 | 0.881 | 0.976 |
| d 12 | 17 | 149 | 169 | 170 | 184 | 299 | 9.2 | 0.001 | 0.001 | 0.001 |
| d 21 | 14 | 102 | 79 | 105 | 102 | 108 | 9.3 | 0.228 | 0.401 | 0.001 |
| d 35 | 92 | 145 | 125 | 146 | 142 | 131 | 9.4 | 0.667 | 0.852 | 0.001 |
| TIBC, μg/dL[6] | | | | | | | | | | |
| d 2 | 203 | 184 | 193 | 190 | 193 | 205 | 17.9 | 0.458 | 0.847 | 0.610 |
| d 12 | 694 | 363 | 402 | 427 | 476 | 648 | 17.6 | 0.001 | 0.001 | 0.001 |
| d 21 | 816 | 411 | 440 | 444 | 419 | 441 | 17.7 | 0.478 | 0.527 | 0.001 |
| d 35 | 540 | 379 | 386 | 404 | 375 | 382 | 17.8 | 0.944 | 0.465 | 0.001 |

[1]A total of 324 pigs (DNA 241 × 600) were used in a 173 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. Day 2 and d 12 represent timepoints in farrowing and d 21 and d 35 represent timepoints in the nursery. Common diets were fed throughout the nursery phase and contained 110 ppm added Fe from $FeSO_4$ from the trace mineral premix.

[2]200 mg of Fe (Gleptoforte, Ceva Animal Health, LLC., Lenexa, KS) administered on d 2, 4, 6, 8, or 10 after farrowing.

[3]Hb = hemoglobin, Hct = hematocrit, and TIBC = total iron binding capacity.

[4]Negative control with pigs receiving no iron injection.

[5]Comparison of d 2 to d 10 injection treatments.

[6]Trt × day interaction ($P < 0.001$).
[7]Represents 2 d after farrowing. Blood was drawn prior to Fe injection.
[8]Represents 12 d after farrowing. Blood was drawn prior to Fe injection.

**Table 2.4 Effects of injectable Fe timing on preweaning and subsequent nursery and growing-finishing growth performance, Exp. 2[1]**

| | | Fe injection day[2] | | | | | Probability, *P* = | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[3] | 0[4] | 1 | 3 | 5 | 7 | 1 and 12[5] | Linear[6] | Quadratic[6] | 0 vs. others[7] | d 1 vs. d 1 and 12[8] |
| BW, kg | | | | | | | | | | |
| d 1 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 1.5 | 0.799 | 0.855 | 0.930 | 0.633 |
| SEM | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | | | | |
| d 19 | 5.1 | 6.1 | 6.1 | 6.1 | 6.0 | 6.1 | 0.146 | 0.607 | <0.0001 | 0.729 |
| SEM | 0.08 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | | | | |
| d 72 | 23.5 | 28.9 | 28.5 | 28.6 | 28.1 | 28.0 | 0.060 | 0.893 | <0.0001 | 0.017 |
| SEM | 0.50 | 0.43 | 0.43 | 0.44 | 0.43 | 0.44 | | | | |
| d 168 | 117.0 | 125.5 | 124.8 | 124.9 | 124.0 | 124.2 | 0.155 | 0.846 | <0.0001 | 0.205 |
| SEM | 1.27 | 1.05 | 1.05 | 1.05 | 1.05 | 1.06 | | | | |
| ADG, g | | | | | | | | | | |
| d 1 to 19 | 192 | 248 | 244 | 247 | 240 | 249 | 0.080 | 0.533 | <0.0001 | 0.699 |
| SEM | 3.7 | 2.8 | 2.8 | 2.8 | 2.8 | 2.8 | | | | |
| d 19 to 72 | 347 | 429 | 424 | 425 | 419 | 413 | 0.104 | 0.947 | <0.0001 | 0.007 |
| SEM | 8.0 | 6.7 | 6.7 | 6.8 | 6.7 | 6.8 | | | | |
| d 72 to 168 | 974 | 1,006 | 1,003 | 1,003 | 998 | 1,001 | 0.361 | 0.857 | 0.001 | 0.539 |
| SEM | 10.6 | 8.8 | 8.8 | 8.9 | 8.9 | 8.9 | | | | |
| d 1 to 168 | 685 | 736 | 732 | 732 | 727 | 728 | 0.154 | 0.837 | <0.0001 | 0.197 |
| SEM | 7.4 | 6.1 | 6.1 | 6.1 | 6.1 | 6.1 | | | | |
| Preweaning mortality, % | 8.0 | 7.4 | 8.6 | 9.8 | 10.0 | 9.0 | 0.181 | 0.725 | 0.676 | 0.445 |
| SEM | 2.07 | 1.42 | 1.53 | 1.62 | 1.65 | 1.57 | | | | |
| Wean-to-finish mortality, % | 2.7 | 2.9 | 2.9 | 2.3 | 5.2 | 4.3 | 0.258 | 0.209 | 0.634 | 0.361 |
| SEM | 1.34 | 1.00 | 1.00 | 0.90 | 1.35 | 1.24 | | | | |

[1]A total of 1,892 suckling pigs (PIC 359 × PIC C40) were used in a 168-d experiment with 11 pigs per sow. Two replications of injectable Fe treatments and 1 replication of the negative control were used within each sow. Pigs were weaned at 19 d after farrowing and placed in a commercial wean-to-finish facility with approximately 125 pigs per pen in a total of 15 pens. Each treatment was equally represented in each pen. Common diets were fed throughout the wean-to-finish phase and contained 110 ppm added Fe from $FeSO_4$ provided from the trace mineral premix.

[2]200 mg of Fe (GleptoForte, Ceva Animal Health, LLC., Lenexa, KS) administered on d 1, 3, 5, and 7 after farrowing.

[3]BW= bodyweight. ADG= average daily gain. Each timepoint represents days after farrowing. Days 1 and 19 represent timepoints in farrowing and d 72 represents timepoints in the nursery.

[4]Negative control with pigs receiving no Fe injection.

[5]Pigs were administered 200 mg of Fe at d 1 and 12 after farrowing.

[6]Comparison of d 1 to 7 injection treatments.

[7]Comparison between mean of pigs receiving no Fe injection and mean of all other pigs.

[8]Pairwise comparison between mean of pigs receiving injection on d 1 and pigs receiving injection on d 1 and 12.

**Table 2.5 Effects of injectable Fe timing on suckling pig hematological criteria, Exp. 2[1]**

| | Fe injection day[2] | | | | | | Probability, *P* < | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item[3] | 0[4] | 1 | 3 | 5 | 7 | 1 and 12[5] | Linear[6] | Quadratic[6] | 0 vs. Others[7] | d 1 vs. d 1 and 12[8] |
| Hb, g/dL | | | | | | | | | | |
| d 19 | 5.0 | 11.1 | 11.3 | 10.7 | 10.3 | 12.2 | 0.001 | 0.113 | <0.0001 | <0.0001 |
| SEM | 0.32 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | | | | |
| Hct, % | | | | | | | | | | |
| d 19 | 15.2 | 32.6 | 33.3 | 31.7 | 30.4 | 36.1 | 0.002 | 0.101 | <0.0001 | <0.0001 |
| SEM | 1.06 | 0.64 | 0.63 | 0.64 | 0.65 | 0.64 | | | | |

[1]A total of 1,892 suckling pigs (PIC 359 × PIC C40) were used in a 168-d experiment with 11 pigs per sow. Two replications of injectable Fe treatments and 1 replication of the negative control were used within each sow.

[2]200 mg of Fe (GleptoForte, Ceva Animal Health, LLC., Lenexa, KS) administered on d 1, 3, 5, and 7 after farrowing.

[3]Hb = hemoglobin. Hct = hematocrit. Day 19 represents weaning.

[5]Pigs were administered 200 mg of Fe at d 1 and 12 after farrowing.

[6]Comparison of d 1 to 7 injection treatments.

[7]Comparison between mean of pigs receiving no Fe injection and mean of all other pigs.

[8]Pairwise comparison between mean of pigs receiving injection on d 1 and pigs receiving injection on d 1 and 12.

# Chapter 3 - Effects of feeding increasing levels of iron from iron sulfate or iron carbonate on nursery pig growth performance and hematological criteria

## ABSTRACT

A total of 140 weanling pigs (241 × 600, DNA, Columbus, NE initially 5.5 ± 0.79 kg bodyweight [BW]) were used in a 32-d study evaluating the effects of increasing dietary Fe from either iron sulfate ($FeSO_4$) or a hydroxylated iron carbonate ($FeCO_3$) on nursery pig growth performance and blood Fe status. The pigs used for this trial did not receive an Fe injection after birth in order to increase sensitivity to added dietary Fe after weaning. Pigs were weaned at approximately 21 d and allotted to pens based on initial BW in a completely randomized block design with 5 pigs in each pen and 4 pens per treatment. Experimental treatments were arranged as a 2 × 3 + 1 factorial with main effects of dietary Fe source ($FeSO_4$ vs. $FeCO_3$) and level (10, 30, or 50 mg/kg of added Fe) plus a negative control with no additional dietary Fe. The basal diet contained 40 mg/kg total dietary Fe based on ingredient contributions and was formulated with an Fe-free trace mineral premix. Experimental diets were formulated below the pigs recommended Fe requirement based on NRC (2012) estimates. Experimental diets were fed in pellet form in a single phase for the duration of the trial. From d 0 to 32, there was no evidence for source × level interactions for growth performance or hemoglobin (Hb) values. There was no evidence for a difference ($P > 0.10$) in dietary Fe source. Providing increasing Fe levels in the diet from either $FeSO_4$ or $FeCO_3$ improved ($P < 0.05$) average daily gain, average daily feed intake, gain-to-feed ratio, and increased ($P < 0.05$) Hb and hematocrit (Hct) values. A day effect ($P = 0.001$) was observed for both Hb and Hct with values increasing throughout the study.

Increasing dietary Fe levels in the diet from either $FeSO_4$ or $FeCO_3$ increased (linear; $P < 0.05$) Hb and Hct values on d 14, 21, and 32. In summary, these data suggest that the micronized form of $FeCO_3$ is a source of Fe that can be added to nursery diets to yield similar responses to those observed from $FeSO_4$ supplementation. Similar to previous research, increasing dietary Fe improved growth performance and increased Hb and Hct values when pigs have low Fe status at weaning.

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

**List of abbreviations:**

ADG, averaged daily gain

ADFI, average daily feed intake

BW, bodyweight

CP, crude protein

DM, dry matter

DMT-1, divalent metal transporter 1

EDTA, ethylenediaminetetraacetic acid

Fe2+, ferrous

Fe3+, ferric

FeCO3, iron carbonate

FeSO4, iron sulfate

G:F, gain-to-feed

Hb, hemoglobin

Hct, hematocrit

# INTRODUCTION

Iron is an essential mineral that is involved in numerous cellular functions such as DNA synthesis and oxidative phosphorylation that are crucial for maintaining normal metabolism. More importantly, Fe plays a critical role in the transport and storage of oxygen (Beard, 2001). To prevent anemia in preweaned swine, an exogenous source of injectable Fe is commonly administered. Injectable Fe has consistently been shown to prevent anemia and support growth in suckling pigs (Peters and Mahan, 2008; Chevalier, 2019). Like the suckling pig, the nursery pig is characterized by rapid growth rate and increased blood volume. The NRC (2012) Fe requirement estimate for nursery pigs from 7 to 11 kg and 11 to 25 kg is 100 mg/kg of the diet to support postweaning growth and blood Fe status. Inorganic Fe sources such as iron sulfate (FeSO4) are commonly added as part of a trace mineral premix to nursery diets and these inorganic sources are routinely provided at or above the NRC (2012) nursery pig requirement estimates (Flohr et al., 2016). The NRC (2012) further suggests that common feed ingredients supplied in the diet contain enough Fe to meet these requirement estimates. However, availability of Fe from these feed ingredients may be limited suggesting the total Fe content of the diet is not reflective of available Fe for the pig (Rincker et al., 2004).

Iron absorption mainly occurs in the lumen of the duodenum. The divalent metal transporter 1 (DMT-1) transport protein is the main facilitator of ferrous (Fe2+) Fe entry into enterocytes of the duodenum (Conrad and Umbreit, 2002). The absorbed Fe is stored within the ferritin molecule in the enterocytes' cytosol or transported via ferroportin into circulation (Xue and Shah, 2013). The transport via ferroportin is regulated by hepcidin, which is a regulatory hormone that helps maintain Fe homeostasis (Ganz, 2013). Inorganic sources of Fe, such as FeSO4, could be of concern as its absorption and availability may be decreased due to

antagonism between trace elements such as copper and zinc (Umbreit, 2005). Iron carbonate (FeCO3; Micronutrients USA, LLC., Indianapolis, IN) is a novel hydroxylated form of FeCO3; thus research is limited on its effects in nursery pigs. Therefore, the objective of this study was to evaluate the effects of increasing dietary Fe from either FeSO4 or FeCO3 on nursery pig growth performance and blood criteria.

## MATERIALS AND METHODS

### *General*

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

### *Animals*

A total of 140 weanling pigs (241 × 600, DNA, Columbus, NE initially 5.5 ± 0.79 kg bodyweight [BW]) were used in a 32-d study. The pigs used for this trial did not receive an Fe injection after birth in order to increase sensitivity to added dietary Fe after weaning. Pigs were weaned at approximately 21 d of age and allotted to pens based on BW in a completely randomized block design to 1 of 7 dietary treatments with 5 pigs per pen and 4 pens per treatment. Dietary treatments were arranged as a 2 × 3 + 1 factorial with main effects of added dietary Fe source (FeSO4 vs. FeCO3) and level (10, 30, or 50 mg/kg) plus a negative control with no added Fe. The Fe sources were substituted for an equivalent amount of corn in the respective diets to form the experimental treatments (Table 1). The FeSO4 source contained 30% Fe and the FeCO3 source contained 37% Fe. The amount of the Fe source added to the diets were adjusted based on the amount of Fe contained in the two sources. Each pen (1.52 × 1.52 m) had metal tri-bar 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 d 0, 7, 14, 21, and 32 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F).

### *Diet Preparation*

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

### *Chemical Analysis*

Complete diet samples were obtained from each dietary treatment during manufacturing. Six individual samples of each dietary treatment were pooled into a composite, subsampled, and sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for analysis of dry matter (DM; AOAC 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).

### *Blood Analysis*

Blood samples were collected via jugular venipuncture in 5-mL ethylenediaminetetraacetic acid (EDTA) and whole blood (Monoject, Covidien, Dublin,

Republic of Ireland) tubes using 22-gauge, 2.54 cm needles from all pigs on day 0, 7, 14, 21, and 32. Hematological criteria measured included: hemoglobin (Hb) and hematocrit (Hct) using an ADVIA 2021i Hematology System (Siemens Healthcare Diagnostics, Tarrytown, NY). Blood samples were processed at the Kansas State University Veterinary Diagnostic Lab located in Manhattan, KS.

### *Statistical Analysis*

Growth data were analyzed as a completely randomized block design using the GLIMMIX procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) with pen as the experimental unit and block as a random effect. Treatment served as the fixed effect in the analysis. The main effects of Fe source and linear and quadratic effects of level, as well as their interactions, were evaluated using preplanned CONTRAST statements. Results were considered significant at $P \leq 0.05$ and marginally significant at $P > 0.05$ and $P \leq 0.10$.

Hematological criteria were analyzed as a repeated measure using the GLIMMIX procedure of SAS 9.4 with pen as the experimental unit and block as a random effect. Treatment served as the fixed effect. Heterogenous variance was applied where appropriate. The main effects of Fe source, day, treatment, and linear and quadratic effects of level, as well as their interactions, were evaluated using preplanned CONTRAST statements. Results were considered significant at $P \leq 0.05$ and marginally significant at $P > 0.05$ and $P \leq 0.10$.

## RESULTS

### *Chemical Analysis*

Results of the diet analysis indicated that all diets had higher Ca compared to the formulated values, while DM and CP closely matched formulated values (Table 2). Iron analysis of the diets indicated that the control diet and diet with 50 mg/kg added Fe from FeCO3 were

higher than expected, with other diets similar to calculated values. Water source used in the experiment originated from a municipal water source which has previously been analyzed and shown to contain low levels of Fe (Williams et al., 2019).

### *Growth Performance*

From d 0 to 32, no evidence of difference ($P > 0.10$) was observed for source × level interactions or source effects for all growth performance criteria (Table 3). Average daily gain, ADFI, G:F and final BW were increased (linear; $P < 0.05$) with increasing Fe from either $FeSO_4$ or $FeCO_3$.

### *Hematological Criteria*

For Hb, there was no evidence of difference ($P > 0.10$) observed for treatment × day interactions (Table 4). For Hct, a marginally significant ($P = 0.089$) source × level interaction was observed on d 21. This interaction was the result of pigs fed diets with 50 mg/kg of added $FeSO_4$ having greater Hct values than pigs fed diets with 50 mg/kg of added $FeCO_3$ on d 21.

Also, there was no evidence of difference ($P > 0.10$) observed for a Fe source effect at any of the blood collection time points for Hb and Hct. A day effect was observed in which all Hb and Hct values increased ($P = 0.001$) throughout the study. For dietary Fe level effect, there was no evidence of difference ($P > 0.10$) in Hb or Hct values on d 0 and 7, but Hb and Hct values increased (linear; $P < 0.05$) with increasing dietary addition of either $FeSO_4$ or $FeCO_3$ on d 14, 21, and 32.

## DISCUSSION

Iron is one of the most abundant trace minerals found in the body of mammals and Hb represents approximately 65% of the Fe found in the body (Munoz et al., 2009). Hemoglobin serves as a transport protein that carries oxygen through the bloodstream from the lungs to

tissues. In young swine, inefficient absorption of Fe reduces the number of circulating red blood cells resulting in Fe deficiency and poor growth performance (Kim et al., 2018). Dietary non-heme Fe is found primarily in the Fe2+ or ferric (Fe3+) forms. The Fe3+ form of Fe has low solubility in the stomach which decreases its availability compared to that of the Fe2+ form (Fuqua et al., 2012). The DMT-1 transport protein is the main facilitator of the Fe2+ form of Fe entry into enterocytes of the duodenum (Conrad and Umbreit, 2002). Once transported to the duodenum, Fe is transferred across the duodenum and transported via ferroportin to red blood cells or bone marrow for erythropoiesis (Xue and Shah, 2013).

Rincker et al. (2004) observed increases in ADG and Hb and Hct values with increasing Fe supplementation from FeSO4 up to 150 mg/kg of the diet. Moreover, Lee et al. (2008) observed similar results where increasing FeSO4 up to 250 mg/kg of the diet increased Hb values in post-weaned pigs. These results suggest that the Fe requirement to optimize growth and hematological criteria is likely above 100 mg/kg of the diet and that the Fe provided in common feed ingredients will not necessarily meet these requirement estimates. Thus, supplemental dietary Fe sources should be added to nursery diets to meet post-weaning Fe requirements for growth and blood Fe status. In our study, we purposely did not provide injectable Fe after birth and fed deficient Fe concentrations in the diet in order to increase the sensitivity of our bioassay.

Research has been conducted to understand if different forms of Fe are more readily absorbed or have increased bioavailability compared to FeSO4. Ettle et al. (2008) observed an improvement in ADG and G:F and increased Hb and Hct values when increasing levels of either Fe-glycinate or FeSO4 were supplemented in nursery diets. Feng et al. (2007) observed no evidence of difference in Hb concentration of nursery pigs when adding 120 mg/kg of Fe from either an Fe-glycine chelate or FeSO4. Novais et al. (2016) observed no evidence for a difference

in growth performance or hematological criteria in nursery pigs when adding 67.5 mg/kg of Fe from FeSO4 or 150 mg/kg from a proteinated Fe. These studies suggest that other Fe sources in addition to FeSO4 are effective at optimizing post-weaning growth performance and blood Fe status. The study herein indicates that the FeCO3 source used is an alternate source of dietary Fe that is as equally effective as FeSO4 to support post-weaning growth performance and blood Fe status similar to previous studies testing other Fe sources.

Iron sulfate and FeCO3 are both non-heme dietary sources of Fe found in the Fe2+ form which would suggest both sources should utilize the same pathway for uptake by the duodenum and have similar bioavailability. Limited research is available on the effects of FeCO3 on nursery pig growth performance and hematological criteria. Ammerman et al. (1974) conducted 2 separate experiments in nursery pigs evaluating different feed-grade FeCO3 sources compared to FeSO4 and observed in both experiments FeSO4 provided the greatest improvements in ADG, Hb concentration, and Hct levels. The results of the study herein would disagree with the observations of Ammerman et al. (1974). The FeCO3 source used in the study herein potentially has improved bioavailability compared to that of the sources used by Ammerman et al. (1974). The improvement in bioavailability potentially could be due to the processes involved in the hydroxylation of the FeCO3 source used in our study. This potentially leads to improved digestion and uptake across the duodenal membrane.

The pigs in the current study would be classified anemic as Hb concentrations at the start of feeding experimental diets were below 10 g/dL (Bhattarai and Nielsen, 2015). Hemoglobin regeneration has been used in Fe depletion-repletion studies to measure the bioavailability of Fe sources compared to that of FeSO4 (Chausow and Czarnecki-Maulden, 1988; Biehl et al., 1997). In the study herein, a day effect was observed as Hb concentrations were increased throughout

the study. This demonstrates regeneration of Hb occurred and pigs improved their blood Fe status with normal feeding behaviors. Because there was no evidence for a difference between FeSO4 or FeCO3 in Hb concentration at any of the collection timepoints, this implies FeCO3 is a suitable source for Fe fortification in nursery diets.

In summary, this study has provided evidence that providing either FeSO4 or FeCO3 in diets fed to pigs that are Fe-deficient elicited similar improvements in nursery pig growth performance and hematological status. Our study also demonstrated that the Fe deficient model utilized was sufficient for evaluating the availability of the 2 different Fe sources and their effects on nursery pig growth performance and hematological criteria. Based on these observations, the data suggests that this micronized form of FeCO3 is a sufficient alternative source of dietary Fe that can be added to nursery diets to meet post-weaning requirements.

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**Table 3.1 Basal diet composition (as-fed basis)**

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

[1] Thermofisher scientific, Waltham, MA.

[2] An Fe-free trace mineral premix (University of Auburn, Auburn, AL) was used to decrease Fe content of the diet.

[3] Corn replaced with an equivalent amount of $FeSO_4$ (Prince Agri Products, LLC., Teaneck, NJ) at 0.07, 0.20, and 0.33% of the diet to form dietary treatments.

[4] Corn replaced with an equivalent amount of $FeCO_3$ (Micronutrients, LLC., Indianapolis, IN) at 0.05, 0.16, and 0.27% of the diet to form dietary treatments.

[6]Standardized total tract digestible phosphorous.

**Table 3.2 Chemical analysis of experimental diets[1]**

| | | $FeSO_4$, mg/kg[4] | | | $FeCO_3$, mg/kg[5] | | |
|---|---|---|---|---|---|---|---|
| Item | Control[3] | 10 | 30 | 50 | 10 | 30 | 50 |
| Dry matter, % | 88.2 | 88.2 | 87.9 | 87.8 | 87.4 | 87.3 | 88 |
| Crude protein, % | 20.8 | 20.9 | 21.7 | 21.7 | 21.5 | 21.7 | 22.0 |
| Ca, % | 0.91 | 0.84 | 0.84 | 0.84 | 0.83 | 0.79 | 0.85 |
| P, % | 0.74 | 0.73 | 0.70 | 0.71 | 0.71 | 0.69 | 0.67 |
| Fe, mg/kg | 50.0 | 55.1 | 72.5 | 87.4 | 46.4 | 67.1 | 109.6 |

[1]An Fe-free trace mineral premix was used in place of normal trace mineral premix to decrease Fe content of diet. Complete diet samples were obtained from each dietary treatment during manufacturing. Six individual samples of each dietary treatment were pooled into a composite, subsampled, and sent to a commercial laboratory (Midwest Laboratories, Inc., Omaha, NE) for analysis of dry matter, crude protein, Ca, P, and Fe in duplicates.

[3]Calculated to contain 40 mg/kg total Fe in the diet.

[4]Iron sulfate (Prince Agri Products, LLC., Teaneck, NJ) added at 10, 30, or 50 mg/kg of Fe.

[5]Iron carbonate (Micronutrients USA, LLC., Indianapolis, IN) added at 10, 30, or 50 mg/kg of Fe.

**Table 3.3 Effects of increasing iron sulfate or iron carbonate on nursery pig growth performance[1]**

| | | | | | | | | | Probability, $P <$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $FeSO_4$, mg/kg[4] | | | $FeCO_3$, mg/kg[5] | | | | | Level | |
| Item[2] | Control[3] | 10 | 30 | 50 | 10 | 30 | 50 | SEM | Source × Level | Source | Linear | Quadratic |
| d 0 to 32 | | | | | | | | | | | | |
| ADG, g | 113 | 190 | 158 | 240 | 179 | 141 | 236 | 22.0 | 0.875 | 0.452 | 0.001 | 0.731 |
| ADFI, g | 203 | 245 | 267 | 298 | 260 | 243 | 307 | 19.7 | 0.952 | 0.972 | 0.001 | 0.944 |
| G:F | 0.549 | 0.752 | 0.628 | 0.798 | 0.686 | 0.588 | 0.754 | 0.052 | 0.925 | 0.185 | 0.014 | 0.846 |
| BW, kg | | | | | | | | | | | | |
| d 0 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 5.5 | 0.01 | 0.200 | 0.474 | 0.938 | 0.358 |
| d 32 | 9.8 | 12.4 | 12.1 | 13.0 | 11.5 | 10.5 | 14.1 | 0.79 | 0.188 | 0.450 | 0.001 | 0.967 |

[1]A total of 140 pigs (DNA 241 × 600) were used in a 1-phase nursery trial with 5 pigs per pen and 4 replications per treatment.
[2]ADG = average daily gain, ADFI = average daily feed intake, G:F = feed efficiency, and BW = body weight.
[3]Formulated to contain 40 mg/kg of total Fe.
[4]Iron sulfate (Prince Agri Products, LLC., Teaneck, NJ) added at 10, 30, or 50 mg/kg of Fe.
[5]Iron carbonate (Micronutrients USA, LLC., Indianapolis, In) added at 10, 30 or 50 mg/kg of Fe.

**Table 3.4 Effects of increasing iron sulfate or iron carbonate on nursery pig hematological criteria[1]**

| | | | | | | | | Probability, $P <$ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $FeSO_4$, mg/kg[4] | | | $FeCO_3$, mg/kg[5] | | | | | | Level |
| Item[2] | Control[3] | 10 | 30 | 50 | 10 | 30 | 50 | SEM | Source × Level | Source | Linear | Quadratic |
| Hb, g/dl[6,7] | | | | | | | | | | | | |
| d 0 | 4.6 | 4.5 | 4.4 | 4.7 | 4.4 | 4.3 | 4.7 | 0.21 | 0.792 | 0.742 | 0.695 | 0.187 |
| d 7 | 4.6 | 4.8 | 4.7 | 5.2 | 4.7 | 4.8 | 4.7 | 0.21 | 0.440 | 0.426 | 0.279 | 0.840 |
| d 14 | 5.1 | 5.5 | 5.3 | 6.1 | 5.3 | 5.5 | 5.7 | 0.23 | 0.635 | 0.595 | 0.006 | 0.522 |
| d 21 | 5.6 | 6.0 | 5.8 | 7.0 | 6.2 | 6.1 | 6.5 | 0.28 | 0.145 | 0.881 | 0.006 | 0.702 |
| d 32 | 6.9 | 7.3 | 7.6 | 8.5 | 7.1 | 7.4 | 8.2 | 0.55 | 0.717 | 0.193 | 0.001 | 0.674 |
| Hct, %[6,7] | | | | | | | | | | | | |
| d 0 | 16.6 | 16.2 | 15.6 | 16.9 | 15.7 | 15.3 | 16.4 | 0.62 | 0.855 | 0.596 | 0.949 | 0.137 |
| d 7 | 16.9 | 17.7 | 16.9 | 19.6 | 17.2 | 17.6 | 17.1 | 0.79 | 0.248 | 0.258 | 0.139 | 0.578 |
| d 14 | 18.5 | 20.3 | 19.1 | 22.3 | 19.6 | 20.0 | 20.8 | 0.85 | 0.588 | 0.647 | 0.005 | 0.630 |
| d 21 | 19.8 | 21.9 | 20.9 | 25.6 | 22.3 | 21.9 | 23.4 | 0.99 | 0.089 | 0.737 | 0.001 | 0.637 |
| d 32 | 24.8 | 26.6 | 26.9 | 29.8 | 26.0 | 26.5 | 29.5 | 1.69 | 0.993 | 0.385 | 0.001 | 0.923 |

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

[2]Hb = hemoglobin and Hct = hematocrit.

[3]Formulated to contain 40 mg/kg of total Fe.

[4]Iron sulfate added at 10, 30, or 50 mg/kg of Fe.

[5]Iron carbonate (Micronutrients USA, LLC., Indianapolis, IN) added at 10, 30, or 50 mg/kg of Fe.

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

[7]Day ($P < 0.001$).

# Chapter 4 - Effects of standardized ileal digestible tryptophan:lysine ratio in diets containing ractopamine HCL on growth and carcass performance of finishing pigs


## ABSTRACT

A total of 1,791 finishing pigs (337 × 1050, PIC, Hendersonville, TN initially 111.2 ± 0.35 kg body weight) were used in a 27-d study evaluating the effects of standardized ileal digestible (SID) tryptophan:lysine (Trp:Lys) ratios in diets containing ractopamine HCL (RAC) on growth performance and carcass characteristics of finishing pigs. Recent research has reported that increasing SID Trp:Lys ratio above 20% in finishing pigs fed RAC resulted in improved growth and carcass performance; however, this response has been inconsistent. Pens of 25 or 26 pigs and 14 pens per treatment were assigned to treatments in a completely randomized block design. The dietary treatments included 5 SID Trp:Lys ratios (20, 22, 24, 26, and 28%). Diets were corn-soybean meal-based and formulated to 0.90% SID Lys. All diets contained 10 mg/kg RAC. Increasing SID Trp:Lys increased (linear; $P < 0.0001$) Trp intake and grams of SID Trp per kg of gain with all pigs consuming over the 4.1 g of SID intake requirement suggested by NRC (2012). For overall growth performance (d 0 to 27), there was no evidence of treatment differences ($P > 0.10$) for average daily gain, average daily feed intake, or feed efficiency. In addition, increasing SID Trp:Lys decreased (linear; $P = 0.002$) carcass yield and tended to increase (linear; $P = 0.078$) backfat depth and decrease ($P = 0.098$) lean percentage. These results suggest that, when finishing pigs are consuming more grams of Trp per day than required by NRC (2012), increasing SID Trp:Lys ratios above 20% elicits no evidence for improvements in growth or carcass performance.



**Key words:** Amino acid, growth performance, late finishing, pig, ractopamine, tryptophan


**List of abbreviations:**

ADG, average daily gain

ADFI, average daily feed intake

BW, bodyweight

CP, crude protein

DM, dry matter

G:F, gain-to-feed ratio

RAC, ractopamine HCL

SID, standardized ileal digestible

## INTRODUCTION

Tryptophan (Trp) is an essential amino acid because of its role in protein metabolism for growing swine. Tryptophan also serves as a precursor for the neurotransmitter serotonin as well as for niacin and melatonin (Moehn et al., 2012). Ractopamine HCl (RAC) is a phenethanolamine β-adrenergic agonist feed additive that repartitions nutrients away from fat deposition towards lean deposition. Ractopamine HCl addition in finishing diets has consistently resulted in improvements in growth rate, feed efficiency, and carcass leanness (Apple et al. 2007; Bohrer et al., 2013). The NRC (2012) standardized ileal digestible (SID) tryptophan to lysine ratio (Trp:Lys) requirement estimate for growing swine from 115 to 135 kg fed 10 mg/kg of ractopamine (RAC) is 18% or 4.1 grams of SID Trp intake per day. Empirical research is scarce on these suggested requirements for growing swine fed 10 mg/kg of RAC. Therefore, the NRC (2012) amino acid profile for growing swine fed RAC are adjusted based on RAC increasing whole-body protein deposition approximately 27% greater than in growing swine fed diets not containing RAC.

Recent research has reported that increasing SID Trp:Lys ratio above 20% in finishing pigs fed diets containing RAC resulted in improved growth and carcass performance. Gonçalves et al. (2018) reported finishing gilts fed diets containing RAC from 106 to 126 kg required a ratio

of 24.5% SID Trp:Lys to provide the 100% maximum response for average daily gain (ADG). Soto (2018a) evaluated increasing SID Trp:Lys from 20 to 28% in finishing pig diets with or without RAC. They observed an interaction in which pigs fed RAC had improved ending bodyweight (BW), ADG, and gain-to-feed ratio (G:F) with increasing SID Trp:Lys from 20 to 28%, while pigs fed diets without RAC had decreased ending BW, ADG, and G:F. However in a follow-up study, the researchers observed that increasing SID Trp:Lys from 20 to 28% in finishing pigs fed diets containing RAC showed no evidence for a difference in growth performance (Soto et al., 2018b).

Due to the inconsistency in response to increasing SID Trp:Lys in diets containing RAC fed to finishing pigs, the objective of this study was to evaluate the effects of feeding high SID Trp:Lys ratios in diets containing RAC on growth and carcass performance of finishing pigs.

## MATERIALS AND METHODS

### *General*

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this experiment. The study was conducted at a commercial research-finishing site in southwest Minnesota using 2 identical barns. The barns were naturally ventilated and double-curtain-sided. Each pen (5.5 × 3.0 m) was equipped with a four-hole stainless-steel feeder and cup waterer for ad libitum access to feed and water. Feed additions were made and recorded by a robotic feeding system (FeedPro; ComDel Innovation, Wilmar, MN).

### *Animals and Diets*

A total of 1,791 finishing pigs (337 × 1050, PIC, Hendersonville, TN initially 111.2 ± 0.35 kg BW) were used. There were 25 or 26 pigs per pen at a floor space of 0.61 m2 per pig, and 14 pens per treatment. Pens of pigs were weighed, and pens were randomly assigned to 1 of

5 dietary treatments in a completely randomized block design by initial average pen BW. The dietary treatments included 5 SID Trp:Lys ratios (20, 22, 24, 26, and 28%; Table 1). All diets were corn-soybean meal-based and formulated to contain 0.90% SID Lys. Nutrient values and SID amino acid coefficients for ingredients from NRC (2012) were used in diet formulation. All diets were formulated to meet or exceed NRC (2012) requirement estimates and contained 10 mg/kg ractopamine HCL (Paylean, 19.84 g/kg; Elanco, Greenfield, IN). Experimental diets were manufactured at a commercial feed mill (New Horizon Farms, Pipestone, MN).

Pens of pigs were weighed and feeder measurements were recorded on d 0, 6, and 27 to determine ADG, average daily feed intake (ADFI), and G:F. On d 6, the three largest pigs per pen were visually selected and marketed following the routine farm protocol. These pigs were included in growth data calculations, but not carcass characteristics. At the conclusion of the trial (d 27), the remaining pigs were given a tattoo corresponding to pen number and were transported to a commercial packing facility (JBS Swift and Company, Worthington, MN) for processing and carcass data collection. Carcass measurements taken at the plant included hot carcass weight (HCW), backfat, loin depth, and percentage carcass lean. Percentage carcass lean was calculated using a proprietary formula using HCW, backfat depth, and loin depth. In addition, percentage yield was calculated by dividing pen average HCW by pen average live weight collected at the research facilities prior to transport to processing facility.

### *Chemical Analysis*

Complete diet samples were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the experiment and stored at -20°C. Diet samples were submitted for total amino acids (method 994.12; AOAC Int., 2012) and Trp (method 13904:2005; ISO, 2005) analysis conducted in duplicate of each treatment by Ajinomoto

Heartland, Inc. All complete diet samples were also analyzed (Ward Laboratories, Kearney, NE) for dry matter (DM; method 935.29; AOAC Int., 2012), crude protein (CP; method 990.03; AOAC Int., 2012), Ca (AOAC 965.14/985.01, 2012), and P (method 968.08 b; AOAC Int., 2012 for preparation using ICAP 6500 [ThermoElectron Corp., Waltham, MA]).

### *Statistical Analysis*

Data were analyzed as a completely randomized block design using the GLIMMIX procedure of SAS (SAS Institute, Inc., Cary, NC). Pen served as the experimental unit and weight block was included in the model as a random effect, which also accounted for barn. Dietary treatments were the fixed effect and initial BW used as a covariate. Preplanned contrasts were utilized to evaluate linear and quadratic effects of increasing SID Trp:Lys. Backfat, loin depth, and percentage lean were adjusted to a common carcass weight using HCW as a covariate. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$.

## RESULTS AND DISCUSSION

Analysis of total amino acid content of experimental diets (Table 2) showed free Trp increased in a step-wise manner with increasing SID Trp:Lys. For overall growth performance (d 0 to 27), increasing SID Trp:Lys increased (linear, $P < 0.0001$) grams of SID Trp intake per day and grams of SID Trp per kg of gain; however, there was no evidence of treatment differences ($P > 0.10$) for ADG, ADFI, G:F, or ending BW (Table 3). For carcass traits, increasing SID Trp:Lys decreased ($P = 0.002$) carcass yield and tended to increase ($P = 0.078$) backfat. Also, lean percentage tended to decrease ($P = 0.098$) with increasing dietary SID Trp:Lys ratios. There was no evidence of treatment differences ($P > 0.10$) for HCW, loin depth, carcass ADG, or carcass G:F.

Tryptophan is considered the second or third limiting amino acid in corn-soybean-meal based-diets fed to growing-finishing swine. Along with serving as a precursor in the formation of serotonin, Trp plays an important role in protein accretion and maintenance functions (Burgoon et al., 1992). Fuller et al. (1989) observed that the optimum Trp:Lys ratio is greater for maintenance than for protein accretion. Therefore, as the pig grows and becomes heavier, the proportion of amino acids going towards maintenance increases relative to that for protein accretion and the Trp:Lys ratio should increase. The NRC (2012) grams of SID Trp intake per day assumed requirement for growing pigs greater than 100 kg is estimated to be 3.0, but because protein accretion rates are greater than that for maintenance, when RAC is fed, the grams of SID Trp intake per day assumed requirement is estimated to be 4.1. For the same weight range, the NRC (2012) suggests the grams of SID Lys intake per day increase from 16.9 to 23.0 when RAC is fed. Although the grams of SID Lys and Trp intake per day increase when feeding RAC, the Trp:Lys ratio is 18% whether or not RAC is included in the diet.

Ractopamine HCl is a phenethanolamine β-adrenergic agonist feed additive that repartitions nutrients away from fat deposition towards lean deposition. Ractopamine HCL inclusion in finishing diets has consistently resulted in improvements in growth rate, feed efficiency, and carcass leanness (Apple et al. 2007; Bohrer et al., 2013). Gonçalves et al. (2018) observed a linear improvement in ADG and G:F up to 24.5% SID Trp:Lys ratio in gilts fed RAC. Soto (2018a) observed that increasing SID Trp:Lys ratio from 20 to 28% of Lys in diets containing RAC also improved growth performance. The discrepancies between our results and those of Goncalves et al. (2018) and Soto (2018a) are likely due to differences in ADFI and grams of SID Trp intake per day. The NRC (2012) grams of SID Trp intake per day requirement estimate for 115 to 135 kg pigs fed 10 ppm RAC is 4.1. Gonçalves et al (2018) observed grams

of SID Trp intake per day that ranged from 2.3 to 4.4 grams while Soto (2018a) observed grams of SID Trp intake per day that ranged from 4.3 to 6.1 grams. The current study observed grams of SID Trp intake per day that ranged from 5.4 to 7.5. However, in the current study, greater ADFI led to a minimum of 5.4 grams per day of SID Trp intake and resulted in no evidence of differences in growth performance with increasing SID Trp:Lys ratios. The results of the current study would agree with observations in a second study from Soto et al. (2018b). They tested SID Trp:Lys ratios from 20% to 28% and observed no evidence for improvements in growth performance. But again with greater ADFI, grams of SID Trp intake ranged from 5.1 to 7.6. The grams per day of SID Trp intake was comparable to the observations in the current study. Therefore, increasing SID Trp:Lys ratios above 20% (approximately 5.4 grams of SID Trp intake per day and higher) in pigs fed RAC did not improve growth or carcass performance.

We observed a significant decrease in carcass yield and percentage lean as well as a tendency for increased backfat depth with increasing SID Trp:Lys ratio. These results are contrary with the results from Soto et al. (2018b) as they observed no evidence of a difference in carcass characteristics with increasing SID Trp:Lys ratios. Soto (2018a) observed a tendency for increased HCW with increasing SID Trp:Lys ratio in finishing pigs fed RAC. In pigs not fed RAC, feeding diets with high levels of Trp have shown no adverse effects on pig performance (Adeola and Ball, 1992; Page et al., 1993). We have no explanation as to why carcass yield and percentage lean decreased as a result of increasing Trp:Lys ratio in this experiment.

In summary, increasing SID Trp:Lys ratio above 20% showed no evidence for an improvement in growth or carcass performance when diets contained RAC. The greater feed intakes resulting in increased SID Trp grams per day of intake observed in this study are in agreement with results observed in Soto et al. (2018b). Feeding above the NRC (2012)

requirement estimate of 4.1 g/d SID Trp in finishing pigs fed RAC did not improve growth or carcass performance.

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**Table 4.1 Diet composition (as-fed basis)**

| Ingredient | % |
|---|---|
| Corn | 75.30 |
| Soybean meal, 47% crude protein | 21.40 |
| Beef tallow | 1.00 |
| Limestone | 0.95 |
| Monocalcium phosphate (21% P) | 0.25 |
| Salt | 0.50 |
| L-lysine-HCL | 0.25 |
| DL-methionine | 0.05 |
| L-threonine | 0.09 |
| L-tryptophan[1] | --- |
| Phytase[2] | 0.02 |
| Vitamin/Trace mineral premix[3] | 0.15 |
| Ractopamine[4] | 0.05 |
| Total | 100.00 |
| | |
| Calculated analysis[5] | |
| Standardized ileal digestible (SID) AA, % | |
| Lysine | 0.90 |
| Methionine:lysine | 32 |
| Methionine and cysteine:lysine | 59 |
| Threonine:lysine | 65 |
| Tryptophan:lysine[1] | --- |
| Valine:lysine | 72 |
| Histidine:lysine | 44 |
| SID lysine:net energy, g/Mcal | 3.54 |
| Net energy, kcal/kg | 2,541 |
| Crude protein, % | 16.8 |
| Calcium, % | 0.50 |
| Phosphorus, % | 0.40 |
| STTD P[6], % | 0.36 |

[1]L-tryptophan added at 0.02, 0.03, 0.05, 0.07, and 0.09% of the diet to achieve SID Trp:Lys ratios of 20, 22, 24, 26, and 28%.

[2]Optiphos 2000 (Huevepharma, Inc., Sofia, Bulgaria) provided 301 FTU per kg of diet and an estimated release of 0.10% STTD P.

[3]Premix provided per kg of premix: 73 g Fe from iron sulfate,; 73 g Zn from zinc sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 220 mg I from calcium iodate; 200 mg Se from sodium selenite; 3,527,399 IU vitamin A; 881,850 IU vitamin D3; 17,637 IU vitamin E; 1,766 mg

vitamin K; 15 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; and 3,307 mg riboflavin.

[4]Paylean (Elanco Animal Health, Greenfield, IN) provided the final diet with 10 mg/kg of ractopamine.

[5]NRC (2012).

[6]Standardized total tract digestible phosphorous.

**Table 4.2 Chemical analysis of experimental diets (as-fed basis)[1]**

| | Standardized ileal digestible Trp:Lys ratio, % | | | | |
|---|---|---|---|---|---|
| Item, % | 20 | 22 | 24 | 26 | 28 |
| Dry matter | 88.7 | 88.5 | 88.8 | 88.4 | 88.3 |
| Crude protein | 16.1 | 16.4 | 16.2 | 16.0 | 16.5 |
| Calcium | 0.64 | 0.60 | 0.60 | 0.57 | 0.60 |
| Phosphorous | 0.37 | 0.38 | 0.37 | 0.36 | 0.39 |
| Amino acids, % | | | | | |
| Lysine | 0.98 | 1.08 | 0.98 | 0.94 | 0.94 |
| Isoleucine | 0.61 | 0.67 | 0.63 | 0.62 | 0.61 |
| Leucine | 1.35 | 1.44 | 1.34 | 1.32 | 1.26 |
| Methionine | 0.35 | 0.36 | 0.33 | 0.31 | 0.31 |
| Methionine and cysteine | 0.59 | 0.62 | 0.57 | 0.55 | 0.54 |
| Threonine | 0.67 | 0.69 | 0.67 | 0.64 | 0.63 |
| Tryptophan | 0.19 | 0.20 | 0.21 | 0.23 | 0.22 |
| Valine | 0.71 | 0.77 | 0.72 | 0.70 | 0.68 |
| Histidine | 0.40 | 0.43 | 0.39 | 0.38 | 0.36 |
| Phenylalanine | 0.82 | 0.88 | 0.81 | 0.78 | 0.74 |
| Free tryptophan | 0.03 | 0.04 | 0.05 | 0.06 | 0.08 |

[1]Diet samples were taken from 6 feeders per dietary treatment 3 d after beginning of the trial and 3 d prior to the end of the trial and stored at -20°C. Amino acid analysis was conducted on composite samples by Ajinomoto Heartland Inc. (Chicago, IL). Samples of the diet were also submitted to Ward Laboratories, Inc. (Kearney, NE) for analysis of dry matter, crude protein, calcium, and phosphorous.

**Table 4.3 Effects of standardized ileal digestible (SID) tryptophan to lysine ratio on growth performance and carcass characteristics of finishing pigs fed Ractopamine[1]**

| | SID Trp:Lys, % | | | | | | Probability, *P* = | |
|---|---|---|---|---|---|---|---|---|
| Item[2] | 20 | 22 | 24 | 26 | 28 | SEM | Linear | Quadratic |
| BW, kg | | | | | | | | |
| d 0 | 110.8 | 111.0 | 111.3 | 111.2 | 111.5 | 0.35 | 0.053 | 0.890 |
| d 27[3] | 135.7 | 135.3 | 136.6 | 135.0 | 137.2 | 0.74 | 0.208 | 0.404 |
| d 0 to 27 | | | | | | | | |
| ADG, g[3] | 980 | 936 | 1001 | 924 | 1011 | 27.0 | 0.521 | 0.199 |
| ADFI, g[3] | 2,674 | 2,633 | 2,725 | 2,605 | 2,687 | 32.1 | 0.978 | 0.753 |
| G:F[3] | 0.367 | 0.356 | 0.369 | 0.354 | 0.377 | 0.0077 | 0.446 | 0.146 |
| SID Trp intake, g/d[3] | 5.4 | 5.8 | 6.5 | 6.8 | 7.5 | 0.08 | 0.0001 | 0.681 |
| SID Trp g/kg gain[3] | 5.5 | 6.2 | 6.7 | 7.4 | 7.5 | 0.13 | 0.0001 | 0.079 |
| Carcass characteristics | | | | | | | | |
| HCW, kg | 100.9 | 100.5 | 100.8 | 100.2 | 100.7 | 1.51 | 0.656 | 0.628 |
| Carcass yield, % | 74.7 | 74.4 | 73.8 | 74.3 | 73.2 | 0.40 | 0.002 | 0.770 |
| Backfat[4], mm | 15.4 | 15.3 | 15.8 | 15.5 | 16.1 | 0.21 | 0.078 | 0.407 |
| Loin depth[4], mm | 73.1 | 73.8 | 73.3 | 73.3 | 73.2 | 0.39 | 0.681 | 0.422 |
| Lean[4], % | 58.3 | 58.4 | 58.0 | 58.2 | 57.8 | 1.43 | 0.098 | 0.308 |
| Carcass performance | | | | | | | | |
| Carcass ADG[5], g | 730 | 695 | 738 | 685 | 741 | 19.2 | 0.838 | 0.176 |
| Carcass G:F[6] | 0.275 | 0.265 | 0.272 | 0.263 | 0.276 | 0.007 | 0.972 | 0.136 |

[1]A total of 1,791 pigs (PIC 1050 × 337) were used with 25 or 26 pigs per pen and 14 replications per treatment.

[2]BW = bodyweight, ADG = average daily gain, ADFI = average daily feed intake , and G:F = feed efficiency.

[3]Adjusted used initial BW as a covariate.

[4]Adjusted using HCW as a covariate.

[5]Carcass average daily gain = overall ADG × carcass yield.

[6]Carcass G:F = carcass ADG/overall ADFI.