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IMPROVED IRON STATUS IN WEANLING PIGS LEADS TO IMPROVED GROWTH PERFORMANCE IN THE SUBSEQUENT NURSERY PERIOD

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THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
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By

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Lexington, Kentucky

2019

ABSTRACT OF THESIS

IMPROVED IRON STATUS IN WEANLING PIGS LEADS TO IMPROVED GROWTH PERFORMANCE IN THE SUBSEQUENT NURSERY PERIOD

The objectives of this thesis were: 1) to assess the iron status of piglets, 2) to thoroughly evaluate the blood profile, growth performance, and tissue mineral concentration of young pigs during the pre and postweaning periods after receiving various dosages of iron (0, 50, 100, 200, and 300 mg iron) at birth, 3) as well as evaluate the effects of an additional iron injection before weaning on hematological measures, growth performance, and tissue mineral concentration postweaning. In the initial experiment, there was a 60% incidence of iron deficiency at weaning after administration of a 150 mg iron injection at birth. Also at weaning, hemoglobin concentration was negatively correlated with BW and BW gain ($r = -0.53$, $P < 0.0001$, and $r = -0.60$, $P < 0.0001$ respectively). In the second experiment, pigs that were not injected with iron at birth had a major reduction in hematological measures, growth performance, and tissue iron concentration until d 52 where iron status was recovered but growth was not. Overall, ADG was improved in a linear and quadratic manner ($P = 0.02$ and $P = 0.01$ respectively) as the iron dosage increased with the largest improvement from the 0 mg to 50 mg iron treatment. The improvement observed in ADG let to similar increases ($P = 0.02$ and $P = 0.01$ respectively) in final BW as iron dosage treatments increased. Hemoglobin (Hb) concentration improved ($P = 0.01$) with increasing injectable iron as early as d 1 and continued to d 38, thereafter (d 52) no differences in Hb concentration were observed. Iron concentration for all tissues (liver, spleen, heart, and kidneys) at weaning was greater ($P \leq 0.01$) as the iron dosage increased. In the third experiment, pigs that were supplemented with an additional iron injection 4 days before weaning had an increased ADG for the overall experimental period (31 to 34 d). The improved ADG during the experiment led to a heavier ($P < 0.001$) final BW (~1 kg) for pigs injected with an additional iron injection. At weaning, pigs injected with a second iron injection had higher ($P < 0.001$) hemoglobin concentration and other complete blood count measures. The improved Hb concentration observed at weaning continued 14 days later ($P \leq 0.02$). Additionally, liver iron concentration was greater ($P = 0.02$) at weaning for the pigs receiving an additional iron injection. In summary, the initial iron injection administered at birth may not be adequate to satisfy all individual iron

requirements of piglets before weaning, however, hematological measurements and tissue iron concentration do improve as the iron dosage increases at birth. Furthermore, injecting an additional iron injection before weaning improves nursery growth performance.

KEYWORDS: Iron deficiency, piglets, weaning, iron injection, growth performance

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12/09/2019

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DEDICATION

To my beloved parents
Janet and Todd Chevalier
For they are the ones who have raised me to be who I am today.

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CHAPTER 1. Introduction

Piglets are born with a very limited iron reserve and obtain only negligible amounts of iron through the sow milk. Traditionally they could obtain this iron requirement through contact with the soil. However, over the past several decades the swine industry has transitioned from more of an extensive production approach such as rearing pigs on pasture to a more intensive production system of raising pigs in a confined, indoor facility. The transition in production methods has led to one of the largest nutritional issues of iron deficiency leading to anemia in modern swine production. Consequently, iron deficiency and anemia have been extensively researched and believed to be preventable through an administration of an exogenous supply of iron usually in the form of an intramuscular injection of 100 to 200 mg iron dextran shortly after birth (NRC, 2012).

However, with modern genetics, increased productivity levels, and rapid growth performances there have been concerns regarding the adequacy of the early-life iron injection. Modern research suggests that hemoglobin is the gold standard indicator of iron status and that optimal levels of hemoglobin concentration (> 11 g/dL) at weaning may lead to improved growth performance in the subsequent nursery period (Gillespie, 2019). Research from the United States, Denmark, and Canada all have shown that following an initial iron injection at birth, there were pigs within a herd at weaning that had hemoglobin concentrations below the optimal level and in some cases severely below (< 8 g/dL) indicating an anemic state (Bhattarai and Nielsen, 2015; Jolliff an Mahan, 2011; Perri et al., 2016). This decreased iron status at weaning following an initial supplement

of iron at birth has been shown to be more applicable to the larger pigs at weaning (Bhattarai and Nielsen, 2015; Jolliff and Mahan, 2011). Pigs with low hemoglobin concentration (< 8 g/dL) at weaning had a reduced BW at 21d-postweaning, in addition, there was a greater incidence of piglets with hemoglobin concentrations in the anemic category 3 weeks postweaning compared to weaning (Perri et al., 2016). The low iron status at weaning can be contributed by many factors such as low birth iron reserves, low iron content in sow milk, rapid weight gain, increased blood volume, etc.; regardless it must be corrected to optimize postweaning health status as well as overall growth performance. Therefore the objective of the present research was to assess the iron status of young pigs from the University of Kentucky swine herd (Chapter 3), evaluate the time course of the blood profile during pre and postweaning periods (Chapter 4), and evaluate the effects of an additional iron injection administered before weaning on postweaning performance (Chapter 5).

CHAPTER 2. Literature review

2.1 Changes and challenges of swine production

The use of contemporary genetic analysis has led to hypotheses that the modern domesticated pig originated from the Eurasian wild boar (Sus scrofa) around 500,000 years ago (Giuffra et al., 2000). Many years after, wild pigs began to become domesticated for a reliable and efficient source of protein. It is thought that domestication occurred around 9,000 years ago (Bökönyi, 1974; Larson et al., 2011). Ever since domestication, pigs have continuously been raised as a source of protein and energy for human consumption. Beginning in the 1920's changes in swine management practices resulted in the start of farrowing sows inside on concrete floors rather than on a pastured or dirt lot. Previous production systems that utilized pastured lots allowed pigs to root through the soil which can be a rich source of minerals, microbes, and other nutrients. This more extensive production system demanded more labor. The change in management systems was thought to be an attempt to increase the efficiency of labor, animal management, animal comfort, and maximize production potential (Cunha, 1977). Shortly after producers realized the benefits of rearing pigs indoors, it became the standard method to produce pigs. Raising pigs in an indoor confinement setting also allowed producers to raise more pigs, completing the transition to a more intensive production approach. From 1977 to 2012, there were strong trends of increasing pig density within herds, which is defined by the increase in average head per operation and decrease in the total operations supporting the transition to a more intensive confinement approach (USDA, 2012). However, the change has led to a large nutritional issue with iron deficiency leading to anemia in modern swine production.

2.2 Iron deficiency anemia

Iron deficiency and anemia associated with iron deficiency are one of the most common nutritional deficiencies found worldwide, and often seen in humans with an inadequate nutrition regimen (Camaschella, 2017). Over the years, iron deficiency anemia has been classified into three stages. First, iron reserves (ferritin) in the liver, spleen, and bone marrow are depleted which leads to a decrease in the ferritin levels in circulating plasma. The second stage, which is defined by a decrease in transferrin (transport protein) saturation, and conversely increasing the expression of transferrin receptors on cells. Finally, due to the lack of transport and supply of iron, the hemoglobin concentration becomes inadequate for the red blood cells making them microcytic and hypochromic (Dallman, 1986). This last stage of iron deficiency is where anemic conditions become prevalent and by this time there is a multitude of problematic issues (i.e. decreased metabolic capacity and immune function). In an anemic state, hemoglobin, hematocrit, and mean corpuscular volume (MCV) are all affected because normal red blood cells are replaced by microcytic and hypochromic red blood cells (Naghii and Fouladi, 2006). Microcytic anemia is the presence of smaller sized red blood cells (Massey, 1992). Hypochromic anemia is when the red blood cells appear less red due to the reduced amount of hemoglobin in the blood cells which contributes to the red color. These two anemia characteristics are often seen together due to the size reduction of the blood cell decreasing the amount of hemoglobin it is capable of carrying (making it paler). Due to the lack of iron carrying capacity of the red blood cells, blood flow is redistributed to the heart and brain at the expense of the other tissues to maintain the

oxygen supply (Dallman, 1986). When anemia becomes more serious and left untreated, other physiological changes develop including the indication of cardiac hypertrophy (Dallman, 1986).

2.2.1 Iron deficiency anemia in swine

Pigs, being biologically similar to humans, also suffer from iron deficiency anemia. Neonatal pigs are the most susceptible to iron deficiency and are the only mammalian species in which neonatal iron deficiency commonly occurs (Szudzik et al., 2018). Iron deficiency anemia in suckling piglets is commonly characterized as hypochromic, microcytic anemia which is similar to human iron deficiency anemia (Szudzik et al., 2018). Iron deficiency anemia in pigs was present in the early 1920s, where early researchers noted the occurrence of anemia when sows were taken off of pasture and placed in a concrete-floored house during farrowing (McGowan and Crichton, 1924). However, earlier production systems that reared pigs outdoors could meet the iron requirement from the pigs rooting the ground. The soil is a substantial source of iron because of the interactions of crystalline iron interacting with plants, microbes, and organic substances making it soluble (Colombo et al., 2014).

2.2.2 Piglets iron requirement

Like all living organisms, pigs need iron. Early work suggests that piglets need around 7 mg of iron per day to refrain from becoming anemic (Venn et al., 1947). It is well understood that piglets have a rapid growth rate, usually gaining several times their birth BW during the first few weeks of life. As a result of this growth, the blood volume of piglets is increased by 30% during the first week of life (Jain, 1986). It also has been found that the heavier or faster-growing piglets had lower hemoglobin and hematocrit

than smaller sized pigs by 17 days of life (Jolliff and Mahan, 2011). The rapid growth rate of young pigs puts a great demand on the erythropoietic system to maintain proper function (Holter et al., 1991). Therefore, taking into account the growth of piglets, work by Braude et al. (1962) estimated that for every kilogram of body weight increase, the piglet must retain 21 mg of iron to maintain a healthy level of body iron. However, later studies indicated that the piglet's requirement for iron during the lactation period can be up to 67 mg of iron for every kg of body weight gain (Kamphues et al., 1992). Conversely, a later study found that piglets needed around 35 to 40 mg of iron for every kg of weight gain in order to maintain normal hemoglobin concentrations (Egeli and Framstad, 1998). While the iron requirement of piglets can vary depending on a multitude of factors (i.e. birth stores, growth rate, etc.), it has been well noted that the iron status of piglets can be associated with weaning weight and postweaning performance. To maximize performance, a growing pig should have blood hemoglobin concentrations over 11 g/dL (Gillespie, 2019).

2.2.3 Assessing iron deficiency anemia in swine

Critical values for assessing iron deficiency can be crucial to producers and veterinarians by identifying early indicators of iron deficiency or disease. However, there can be a great amount of variation and the reference values most likely are dependent on the life stage of the pig often making them misleading. In piglets, iron deficiency anemia is most commonly assessed using hemoglobin concentration. Clinical iron deficiency anemia is defined as hemoglobin less than 9 g/dL, sub-clinical iron deficiency anemia if hemoglobin concentration is between 9 to 11 g/dL, and normal if it is greater than 11 g/dL (Von der Recke and Heisel, 2014; Fredericks et al., 2018; Gillespie, 2019). Another

common method of evaluating iron status is through a complete blood count (CBC). A complete blood count measures hemoglobin concentration (Hb), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Hemoglobin concentration is the total amount of hemoglobin in the blood while RBC and WBC are the number of red and white blood cells in a given concentration of blood, respectively. Hematocrit is the fraction of blood that is made of red blood cells. Mean corpuscular volume is a measure that indicates the size of the red blood cell. Mean corpuscular hemoglobin is the amount of hemoglobin in red blood cells compared to MCHC which is the amount of hemoglobin relative to the size of the red blood cell (Sarma, 1990).

2.3 Maternal iron contribution to piglets

2.3.1 Fetal iron development

The fetus lives in the maternal uterus for around 114 days where it will undergo many changes. During this time, the fetus is relying solely on the sow to provide it with many nutrients, including iron that it requires following birth. Iron has to be transported across the maternoplacental barrier via endometrial secretions of uteroferrin (Renegar et al., 1982; Roberts and Bazer, 1988). Uteroferrin is a glycoprotein that contains and transports iron from the uterus to the developing fetus (Bazer et al., 1975; Roberts and Bazer, 1980). However, the transfer of large molecules like glycoproteins across the placenta is limited in the pig (Hemmings and Brambell, 1961). Iron can also be transferred directly to the fetus by blood through the epitheliochorial placenta, but the rate is also very limited (Douglas et al., 1972). Iron accretion in the developing fetus has been observed to

increase with gestational age. Mahan et al. (2009), found that iron deposition had a quadratic increase from 45d post-conception to birth and the largest increase occurred during the last 15 d of gestation. Although the body composition of an individual piglet will vary, the amount of iron that piglets are born with is estimated to be around 50 mg of iron (Venn et al., 1947). In newborn pigs, the body iron reserves are largely dependent on the litter size of the sow. An increase in litter size causes a reduction in body iron in individual fetal pigs (Mahan et al., 2009). Physiologically, it is difficult for the sow to adequately distribute iron to each individual fetus (Svoboda and Drabek, 2005).

There have been several attempts to increase fetal iron status through the nutrition of the sow. However, manipulating the diet of the sow by supplementing organic or inorganic iron or increasing dietary iron levels has been very inconclusive in terms of altering fetal iron status. Piglets from sows fed an organic source of iron (chelated to hydrolyzed soy protein, Bioplex TM premix) had significantly lower hemoglobin levels at birth and 2 days following birth in comparison to sows fed an inorganic source of iron (salt form as ferrous sulfate) (Peters and Mahan, 2008). In disagreement, more recent work demonstrated that increasing the dietary level of iron using an organic source (ferrous glycine chelate) was found to increase organ weights and hematological parameters of neonatal piglets compared to an inorganic source of iron (ferrous sulfate) (Li et al., 2018).

Affecting the iron reserves of fetal piglets could involve more than simply altering the dietary level of iron for a sow. Although it is clear that many nutrients cross the epitheliochorial placenta from the sow to the developing fetuses, the past research

suggests that there may be an insufficiency of the molecular mechanism for iron transport (Szudzik et al., 2018).

2.3.2 Sow milk

Once born, sow colostrum and milk are the sole source of nutrients for piglets. Depending on the litter size it is estimated that a nursing pig only receives around 1 mg of iron per day from maternal milk (Venn et al., 1947). However, as litter size increases, the amount of milk and iron per pig decreases. Compared to other nutrients in the milk, the concentration of iron is minimal. Table 2.1 represents some of the macro and micro mineral contents of sow milk found throughout the literature. The mineral concentration of the sow milk is typically known to increase gradually until week two post-partum, where it then remains constant (Hurley, 2015). There have been numerous studies investigating milk composition of sows fed supplemental dietary iron; however, the effects of iron concentration were minuscule (Venn et al., 1947; Pond et al., 1965; Veum et al., 1965). In one scenario, dietary supplementation of ferrous sulfate at an inclusion rate higher than the NRC estimates (120 mg/kg Fe vs. 80 mg/kg Fe) led to a decline in iron content of the milk during the lactation period compared to the NRC amount (Wei et al., 2005). It has also been found that high lactating sows (that produce faster-growing piglets) had a lower milk iron content compared to sows with lower milking production, indicating that high milk production may dilute the iron concentration of the milk (Elliott et al., 1971).

Table 2. 1 Composition of sow milk at different times of lactation

Milk sample[1]	Macro-minerals mg/kg milk Ca	P	Micro-minerals mg/kg milk Fe	Zn	Cu	Reference
Lactation average[2]	1272.8	1133.4	2.3	9.2	2.7	Csapo et al., 1995
21 d post-partum[2]	2233.0	1509.0	2.2	7.5	0.8	Coffey et al., 1982
Colostrum[3]	-	-	1.7	7.3	2.1	Lu, 2018
Colostrum[3]	887.5	1352.5	1.8	15.0	2.6	Peters et al., 2010
17 d post-partum[3]	1945.0	1470.0	1.7	7.1	0.5	Peters et al., 2010

[1]Milk sample represents the time at which milk was collected and analyzed.
[2]Means reported on a DM basis.
[3]Means reported on a wet basis.

2.4 Swine management practices

2.4.1 Older weaning ages

The age at weaning can be crucial to subsequent growth and health status of piglets in the nursery and finishing stages. When the average weaning age was increased from 15 to 20 days, the later weaning age pigs had an increase in ADG and 42 d BW ($P < 0.01$ and $P < 0.001$; respectively), as well as a lower morbidity occurrence (1.01% vs. 2.07%) (Smith et al., 2008). However, increasing weaning age too much can have a negative impact on the hematological status of piglets due to the rapid growth rate and limited supply of iron during the lactation period. Work from the European Union, which commonly practices a later weaning age demonstrated that larger or faster-growing pigs at a later weaning age (~25d) had decreased hematological indices compared to smaller pigs (Bhattarai and Nielsen, 2015).

2.4.2 Increased sow productivity (genetics)

Over the years, sows have continuously been genetically selected for high productivity. Sow productivity can be defined by many characteristics, however, the main

assessment is the number of pigs weaned per litter, per year. The United States, behind China, and the European Union is the third-highest pork producing region in the world with around 11% of the total global production (FAS, 2019). Sow productivity in terms of pigs weaned per litter has seen a 21% increase from 2004 to 2019 (Table 2.2). Also, the average total of pigs per litter in the United States has increased from 11.5 in 2004 to 14.6 in 2019 (Pig Champ, 2019).

Table 2. 2 Sow productivity improvement in the United States over the years[1]

	Year			
Items	2004	2010	2018	2019
Total pigs per litter	11.5	12.8	14.5	14.6
Pigs born alive/litter	10.3	11.5	13.0	13.1
Pigs weaned per litter	9.1	10.2	11.3	11.3
Weaned pigs/sow/year	21.3	23.4	25.3	25.7
Average age at weaning	18.2	20.1	20.5	20.8

[1]Cited from Pig Champ (2019).

2.4.3 Iron supplementation in swine production

Ever since early reports of the occurrence of iron deficiency when pigs were raised on concrete floors, iron supplementation has always been part of normal production practices. However, the manner and amount of supplemental iron are highly variable and dependent on the individual farm and their labor situation. It is very common to supplement the piglets with 100-200 mg iron by IM injection within the first few days after farrowing (Almond et al., 2017). It is also recommended by the NRC (2012) to provide a single dose of 200 mg iron to pigs shortly after birth to prevent iron deficiency anemia. When pigs were injected with increasing levels of gleptoferron (0, 50, 100, 150,

and 200 mg Fe) at d 3 post-partum there was a linear increase in ADG for the 21 d lactation period (Williams et al., 2018). Gleptoferron is similar to iron dextran as it is a macro-molecule complex that contains iron.

2.5 Current issues in the swine industry

In today's swine industry, pigs have been continuously selected for high performance resulting in a rapid growth rate following birth. It is thought that pigs that undergo a more rapid growth rate in the nursing phase are at a greater danger of becoming iron deficient and even anemic (Svoboda and Drabek, 2005). Assessing the iron status of piglets at weaning (17 days of age) showed that as body weight increased both hemoglobin and hematocrit decreased (Jolliff and Mahan, 2011). Subsequently, a study involving pigs from 11 different farms that administered 200 mg of iron to pigs found that 75% of the pigs were either sub-clinically (Hb = 9-11 g/dL) or clinically iron-deficient anemic (Hb < 9 g/dL) around weaning (Von der Recke and Heisel, 2014). A report from 5 commercial Danish farms showed that larger pigs had lower ($P < 0.05$) serum-Fe than smaller pigs (Bhattarai and Nielsen, 2015). More recent work in Canada, also found that larger pigs at weaning had lower hematological measures (Perri et al., 2016).

Iron deficiency at weaning can be exacerbated because of the weaning stress and cause an "iron gap". An iron gap occurs when faster-growing piglets reach low hemoglobin concentrations before weaning and it gets lower with the weaning transition stress (Gillespie, 2019). Weaning pigs with a low iron status can be costly in the subsequent growing periods. Pigs that were classified anemic (Hb < 9 g/dL) at weaning were 0.82 kg lighter at 21d-postweaning than non-iron deficient pigs (Perri et al., 2016). Under Norwegian production conditions (later weaning age), piglets with access to a high

iron creep feed had relatively low hemoglobin concentrations around 21 and 35 days (10.2 and 10.1 g/dL, respectively) after birth which was later confirmed by later studies (Egeli and Framstad, 1998; Egeli et al., 1998). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were all declining from 17 to 21 days after piglets received an initial iron injection (180 mg Fe) after birth (Holter et al., 1991). Holter et al. (1991) also reported that pigs administered the iron at birth had smaller erythrocytes on d 21, suggesting that an iron injection given at birth may be insufficient to sustain normal production of erythrocytes limiting hemoglobin synthesis after 21 days.

Although pigs were administered 200 mg iron shortly after birth in the forms of iron dextran and gleptoferron, Morales et al. (2018) observed a decrease in both serum iron and serum ferritin from days 14 to 17, subsequently, serum ferritin also decreased from 17 to 21 days of age. This data possibly suggests that an initial 200 mg iron injection given at birth only supplies iron to the pig until 14 to 17 days of age. Van Gorp et al. (2012) estimated that a single iron injection (200 mg iron) will only cover approximately 4 kg of growth for a suckling pig. Furthermore, they proposed a theoretical model that estimated 390 mg iron would be needed to prevent a pig from becoming iron deficient before weaning. Under these assumptions, it is hypothesized that piglets that grow faster will fall into a period of iron deficiency, in which the total weight gain exceeds the available iron reserves (Van Gorp et al., 2012). This period of iron deficiency often comes around the weaning time which can escalate the problem because of the low feed intake by the pig due to the stress associate with the first several days after weaning.

However, optimizing iron status can have beneficial effects. At weaning, pigs classified in the optimal hemoglobin range (> 11 g/dL) had a higher ($P < 0.05$) body weight at 8 weeks postweaning compared to pigs from the sub-clinical anemia (9-11 g/dL) and clinical anemia (<9 g/dL) status categories (Fredericks et al., 2018). Increasing hemoglobin levels to reach an optimal range is thought to have positive effects on the growth performance and overall well-being of piglets because of the potential to improve oxygen transport, immune function, and metabolism support (Von der Recke and Heisel, 2014). Iron status can also have other added benefits other than its role in the erythropoietic system. Pigs that had higher hemoglobin concentration at 4 weeks of age had a higher energy intake as well as energy retention compared to pigs which had lower levels of hemoglobin concentration (Gentry et al., 1997).

2.6 Addressing the iron issue

2.6.1 Greater initial dose of iron

Attempting to correct for low iron status in the pre-weaning period, it is logical to increase the dosage of the iron injection at processing. Research implementing this strategy demonstrated that increasing the initial iron injection from 200 mg to 300 mg of iron resulted in no detrimental effects, numerical increases in hematological status, but no effects on growth performance (Murphy, 1997). Similar work also showed that pigs receiving 300 mg compared to 200 mg of iron shortly after birth only had minor numerical increases in hematological indices but no response in growth performance through 4 weeks of age (Gaddy et al., 2012).

2.6.2 Addition of a second iron injection

Another attempt to correct low iron status at weaning and maximize growth performance in the subsequent growing period is to give an additional iron injection. Early work showed that pigs that received an additional 200 mg iron injection at d 21 of life had 4.3% higher weight gain than pigs not receiving the second injection. Once weaned at 28 days, the pigs injected twice had an 8% higher weight gain than those with only one injection during a 3 week nursery period (Kamphues et al., 1992). Later, it was demonstrated that pigs weaned at 34 days of age that received an additional 200 mg iron injection at d 20 of lactation led to higher hemoglobin levels and a 6% increase in ADG for the first 15 days of the nursery (Haugegaard et al., 2008). Pigs receiving 200 mg of iron at birth compared to pigs receiving 200 mg of iron at birth plus an additional 100 mg of iron at 10 d of age had higher ($P < 0.01$) hemoglobin and hematocrit levels by 17 days of age (Jolliff and Mahan, 2011). The addition of a second 100 mg iron injection before weaning resulted in numerically higher feed intake and ADG during the first three weeks of the nursery which led to a slightly heavier final BW of 0.7 kg heavier (Jolliff and Mahan, 2011). Agreeing with the previous research, the addition of a second 200 mg iron injection improved ($P < 0.01$) hemoglobin concentrations but there were no differences ($P > 0.05$) observed between treatments on ADG (Perrin et al., 2016). Another study also demonstrated an increase ($P < 0.05$) in hemoglobin concentration at 21 and 35 days of age for pigs receiving an additional iron injection at d 11 compared to pigs only receiving one iron injection on d 3 (Williams et al., 2018). On the other hand, administering a second iron injection at weaning only improved the ADG of pigs that were classified as larger (weaning BW > 6 kg), in comparison to the larger pigs that only received the initial

iron injection at processing (Urbaniak et al., 2017). Almond et al. (2017) reported in a case study that a farm that used a two-injection approach had greater weight gains through 30 days of the experiment compared to the farms that only used a single injection. More recently, pigs that were supplied 200 mg iron at birth and another 200 mg at processing (5-7d) had higher ($P < 0.05$) hemoglobin concentration at weaning compared to pigs receiving only a single 200 mg or pigs receiving 100 mg at birth and 100 mg at processing (Fredericks et al., 2018). It is thought that using a second injection optimizes hemoglobin and iron status of the pigs which could possibly promote peak immunity or an increased health status (Perrin et al., 2016). This was supported in earlier work, where pigs under positive disease conditions (postweaning multisystemic wasting syndrome or PMWS), that were given a second iron injection five days before weaning (~28d), had an ADG of 50 g/d more than the single injected piglets (Bach, 2006).

An explanation for the efficacy of a second iron injection can be explained with the "iron gap" concept. The iron gap is when the iron stores from the initial injection at processing and the iron that pigs are born with start to become depleted before they receive and consume adequate amounts of a diet supplemented with iron after weaning (Von der Recke and Heisel, 2014; Gillespie, 2019). This situation is exacerbated further depending on the length of the nursing period and the weaning transition where feed intake declines tremendously. Research that developed a model to estimate the economic impact of administering a second iron injection to maximize the hemoglobin status of weanling pigs to optimize health demonstrated an incredible potential to add value to the current swine industry (Olsen, 2019). Olsen (2019) estimates that even with current industry iron injection practices, the total economic impact of sub-clinical and full-scale

iron deficiency anemia in the United States swine herd ranges from 46.3 to 335.7 million US dollars.

2.7 Postweaning iron supplementation

The latest edition of the NRC estimates that a growing pig (5 to 25 kg) requires 100 mg/kg Fe included in the diet (NRC, 2012). The previous version of the NRC only estimated that growing pigs (10 to 20 kg) required 80 mg/kg in the diet (NRC, 1998). The increased estimate from 1998 to 2012 may be explained by the increased incidence in iron deficiency and research regarding dietary iron. Research by Rincker et al. (2004) reported that feeding increased supplemental dietary iron (0, 25, 50, 100, and 150 mg/kg iron) resulted in a tendency to improve ADG and ADFI ($P = 0.08$ and $P = 0.09$, respectively) for the 35 d nursery trial. Additionally greater ($P < 0.05$) hemoglobin and hematocrit values were observed on d 21 and 35 with increasing supplemental dietary iron (Rincker et al., 2004). Another experiment that fed weaning pigs increasing levels of dietary supplemental iron (0, 80, and 160) also showed improved ($P < 0.05$) ADG but no effect on ADFI during a 35 d nursery study (Jolliff and Mahan, 2011). Although it is recommended to supplement dietary iron in the diet of nursery pigs, there may already be sufficient levels of iron within the ingredients used in the diet. Rincker et al. (2005) analyzed individual dietary ingredients and found high levels of iron present in common nursery diet ingredients like mono and dicalcium phosphate, limestone, and fishmeal (8941, 7741, 425, and 705 mg/kg iron, respectively).

2.8 Nutritional iron

Nutritional iron and its relation to iron deficiency date back to the 1700s when it was observed that people who were pale and listless would mix rust with a drink which would

restore their health (Carpenter, 1990). In 1830, Professor Pierre Blaud recommended taking ferrous salts to aid in chlorosis (Carpenter, 1990). After many attempts to disprove the efficacy of inorganic iron, work by Stockman (1893) proved that inorganic iron is absorbed and seemed to be utilized more readily than organic iron. The importance of iron was finally made clear through convincing evidence that supported inorganic iron was needed for hemoglobin synthesis (Yip et al., 1996). Since that time iron has been well accepted as a biologically essential element for every living organism (Aisen et al., 2001; Lieu et al., 2001).

2.8.1 Iron storage

Iron is mainly stored in the body by storage proteins (ferritin and hemosiderin) located in the liver, reticuloendothelial cells, spleen, and bone marrow (Dallman, 1986; Massey, 1992). Ferritin is a storage protein that protects iron from the redox potential by a chaperone protein, poly (rC)-binding protein (PCBP1) (Lieu et al., 2001; Camaschella and Pagani, 2018). Ferritin consists of the ferric form of iron however, to be released for bodily functions, ferric iron (Fe^{3+}) is reduced to ferrous iron (Fe^{2+}) (Casiday and Frey, 1998). Approximately 25% of iron in the body is accounted for in mobilizable iron stores (Trumbo et al., 2001). Storage sites of iron can be almost completely depleted before iron deficiency is noticed; in contrast, a 20-fold increase of iron stores may occur before there is evidence of iron overload (Dallman, 1986).

2.8.2 Iron transport

In the body (plasma and tissues), virtually no iron is in the free ionic form (Strain and Cashman, 2002). Free ionic iron acts like other free radicals which can cause oxidative reactions resulting in damage to tissues. Due to the high occurrence of protein

sequestering of iron, the rate of oxidative damage to biomolecules is limited (McAnena, 2005). These proteins that bind to iron prevent oxidative damage but are also responsible for the transport, storage, and homeostasis of iron in the body.

2.8.2.1 Transferrin and ferroportin

Transferrin is a protein carrier responsible for carrying two atoms of ferric iron through extracellular spaces from the reticuloendothelial system and the small intestine to the bone marrow for the synthesis of hemoglobin in developing red blood cells (Dallman, 1986; Toblli and Angerosa, 2014). Transferrin delivers iron at a rate which is dependent upon the amount of mono- and di-ferric transferrin, as well as the frequency of red blood cell production (Huebers and Finch, 1984). Transferrin carries ferric iron across the target cell's membrane where it is then released (Munoz et al., 2009). Once ferric iron is inside the cell, ferric reductase then reduces it to the ferrous state, allowing it to be transported to the cytoplasm by DMT-1 (Munoz et al., 2009). Ferroportin expression is also vital in the transportation of iron for the regulation of homeostasis via its ability to traffic iron into circulating pools from enterocytes and macrophages (Wessling-Resnick, 2010).

2.8.3 Role and function in living organisms

Iron can alternate between the divalent and trivalent states, this property is what allows it to be so beneficial for living organisms. Ferric iron is the oxidized state of iron (Fe^{3+}) whereas ferrous iron is the reduced state (Fe^{2+}). The alternation between ferric and ferrous states, act as the functional basis for protein binding and other physiological functions such as the redox reactions in which iron participates (McAnena, 2005).

2.8.3.1 Erythropoiesis

Erythropoiesis is the development of red blood cells (erythrocytes) (Beckman et al., 2010). Erythrocytes make up the majority of the cell types in blood. Erythrocytes are first produced in fetal animals by differentiation of erythro-myeloid progenitors in the yolk sac and fetal liver (Dzierzak and Philipsen, 2013). The iron accumulated during pregnancy in the fetal liver is the main source of iron used for the early stages of erythropoiesis by the fetus (Rao and Georgieff, 2007). However, at the time of birth the site of erythropoiesis switches from the liver to the bone marrow and spleen (Dzierzak and Philipsen, 2013). In the case of immature or young pigs, early-in-life erythropoiesis can occur extramedullary pushing the process to the liver and spleen (Beckman et al., 2010).

2.8.3.2 Hemoglobin and myoglobin

Hemoglobin represents more than 65% of the iron found in the body and functions as the transport protein that carries oxygen from the lungs to the tissues via the bloodstream (Dallman, 1986; Munoz et al., 2009). The heme portion of hemoglobin contains iron which acts as a coordinating ion and binds to molecular oxygen (Casiday and Frey, 1998). Iron combines with a protoporphyrin to make up the heme polypeptide. Hemoglobin is then made up of 4 heme polypeptide chains that interlock and make a globular protein (Ali, 1976).

Myoglobin represents about 10% of body iron and is the red pigmentation in muscle. It is responsible for the transport and storage of oxygen during muscle contraction (Dallman, 1986). Similar to hemoglobin, myoglobin transfers the oxygen from hemoglobin to muscle cells and cytochromes which are used for energy (Casiday and

Frey, 1998). Although similar to hemoglobin, myoglobin is comprised of only 1 heme polypeptide chain (Kendrew et al., 1960).

2.8.3.3 Energy metabolism

In the body, iron also exists as iron-sulfur clusters, which play a vital role in energy metabolism. These non-heme iron compounds such as nicotinamide adenine dinucleotide (NADH) dehydrogenase, succinic dehydrogenase, and xanthine oxidase, can account for more iron present in the mitochondria than cytochromes (Dallman, 1986). Cytochromes are similar in structure (one atom of iron) to myoglobin, they are enzymes found in the mitochondria and are essential for the production of adenosine triphosphate (ATP) (Dallman, 1986). The major cytochrome associated with iron is ferricreductase (Dcybt).

2.8.4 Bioavailability

2.8.4.1 Absorption and utilization

Work by Conrad et al. (2000) showed that there are two separate independent pathways for transport and uptake of ferric and ferrous iron. Ferric iron is absorbed by a B3 integrin and mobilferrin pathway (IMP) that is independent of any other minerals, in contrast to ferrous iron, which is regulated by the shared divalent metal transporter-1 (DMT-1) (Conrad and Umbreit, 2002). However, due to the continuous growth of the gastrointestinal tract of the young pig, it is well thought that the expression of DMT-1 is relatively low (Svoboda and Drabek, 2005). Iron absorption into the body's circulation requires the passage through the apical membrane, by translocation through the cytosol, and the export across the basolateral membrane (Lieu et al., 2001). Absorption occurs in the proximal small intestine (duodenum) (Conrad and Umbreit, 2000). It is also thought

that the enterocytes present in the crypts of the duodenum absorb iron from the plasma (Munoz et al., 2009).

2.8.4.1.1 Dietary absorption

Dietary iron is made up of about 10% heme iron and 90% non-heme iron which rely on different mechanisms for absorption (Munoz et al., 2009). Heme iron is soluble at intestinal pH and is not influenced by dietary constituents (Conrad and Umbreit, 2000). Heme iron precipitates under acidic conditions and is absorbed through a heme carrier protein (HCP1) which is responsible for transporting heme across the apical membrane of the duodenal epithelial cells (Krishnamurthy et al., 2007). After globin degradation by the pancreatic enzymes, heme enters the intestinal absorptive cell as a metalloporphyrin and is not competitive with non-heme iron (Conrad and Umbreit, 2000). Next, the absorptive cell releases inorganic iron from the porphyrin ring by heme oxygenase (Raffin et al., 1974). Once released by the absorptive cell, iron from the heme source and non-heme iron compete for transfer from the cell into the circulating plasma (Conrad and Umbreit, 2000).

Non-heme iron is less available for absorption due to the high oxidation rate of iron (II) to iron (III) (Spiro et al., 1967). Dietary non-heme iron is primarily in the form of ferrous salts, which usually oxidizes to the ferric form under the acidic conditions found in the stomach and duodenum rendering it insoluble (Conrad and Umbreit, 2000). Absorption of non-heme iron is most prevalent in intestinal villi as the soluble ferrous ions are low-molecular-weight ligands which are facilitated by the acidic conditions of the stomach (Hunt, 2005). However to become available for absorption the ferric state of iron has to be reduced to ferrous through the Dcytb or chelated before DMT-1 moves it

across the intestinal epithelium (Munoz et al., 2009). DMT1 transports iron across the electrochemical gradient and apical membrane of the enterocytes through a proton cotransport mechanism (Pietrangelo et al., 1992; Fleming et al., 1997; Shawki et al., 2012).

2.8.4.1.2 Intramuscular injection absorption

Early work found that anemic piglets utilized 93% of the dose of iron dextran by 14 days after intramuscular injection, suggesting that an intramuscular injection has a high and rapid absorption rate (Martin et al., 1955). After administration of an iron-carbohydrate complex (iron dextran), the complex mixes with plasma and enters the reticuloendothelial system (RES) via an intravascular fluid compartment (Danielson, 2004). Once in the RES, phagocytes from the liver, spleen, and bone marrow collect the iron agent and release it from the iron-binding compound (Danielson, 2004). Ferrous iron is cleaved via the endosome fusing with the lysosome creating an acidic and reducing environment allowing DMT-1 to transport it across the endolysosomal membrane to enter the iron pool found in the macrophage cytoplasm (Geisser and Burckhardt, 2011). Microscopic analysis of liver sections showed heavy non-heme iron accumulation in the liver of pigs as soon as 5 days after injection of iron dextran, however, the iron deposits decreased as piglet body weight increased (Pu et al., 2018).

2.8.5 Regulation and homeostasis

Iron homeostasis is tightly regulated due to the toxicity and cell death from free radical formation and lipid peroxidation that is associated with iron overload (Britton et al., 1994). Iron is highly recycled and is negligibly excreted through major bleeding, urination, defecation, and sloughing of skin cells (Casiday and Frey, 1998). Because of

this, the absorption of dietary iron is strictly regulated on the basis of homeostasis within the body (Siah et al., 2006). To maintain iron homeostasis, iron uptake, transport, storage, and utilization is all controlled by intracellular iron levels through a feedback regulatory mechanism that uses specific mRNA-protein interactions in the cytoplasm (Lieu et al., 2001). For example, mucosal absorption of iron from the lumen is regulated by the concentration of iron within the absorptive cell (Conrad and Umbreit, 2000). For the regulation of circulating iron in the blood, ferritin can release iron when there is a shortage of circulating iron and can also store iron when there is an abundance (Casiday and Frey, 1998). Tight regulation of iron homeostasis was supported in work showing that increasing dietary iron intake can increase overall iron absorption, however it reduces the efficiency of iron absorption (Werner et al., 1982).

2.8.5.1 Iron toxicity

Iron toxicosis is not a common problem seen in most domestic animals as iron is highly regulated and absorption is dependent on the need of the animal. Nonetheless, in extreme scenarios where iron is in abundance, signs of iron toxicosis can occur when an iron overload of tissues and iron-binding capacity is exceeded, allowing free ionic iron to cause peroxidative damage in tissues and membranes (NRC, 2005). The NRC (2005) has set a maximum tolerable level of dietary iron at 3000 mg/kg for swine which is defined as the dietary level that when fed for a defined period of time will not impair accepted indices of animal health and performance. However, most iron toxicosis research is focused on orally or dietary amounts of iron in contrast to parenteral iron supplementation such as an intramuscular (IM) injection.

There is a large concern that high levels of injectable iron may influence bacterial growth. Ku et al. (1983) found that after administering a 150 mg intramuscular (IM) iron injection to piglets, serum iron drastically increased by 6 hours and peaked at 24 hours where it then declined sharply at 4 days suggesting that iron is well absorbed after an IM injection and is removed from the serum about 1 day after the injection where it is readily available for hemoglobin synthesis or spleen and liver iron storage. Furthermore, work by Knight et al. (1983) demonstrated that after an IM injection of either 100 or 200 mg iron as iron dextran, serum iron peaked at 8 hours for the 100 mg iron treatment in contrast to the 200 mg iron treatment which peaked at 16 hours. More importantly, there was a clearance of 90% of the peak serum iron concentration by d 2 and 4 respective of the treatment, suggesting that the rate of serum iron clearance is dependent on the amount of iron injected. In a different experiment looking at the relation of serum iron and bacterial growth, Knight et al. (1983) reported that pigs injected with 200 mg iron dextran exhibited much higher ($P < 0.01$) E. coli growth in the serum only on day 1 after injection compared to serum from pigs injected with 100 mg iron dextran. On days 3, 5, 7, 9, and 11 there were no differences in serum bacteria growth between the 100 and 200 mg iron dextran treatments.

Lipiński et al. (2010) showed that piglets that were supplemented with high levels of iron dextran (100 mg Fe/ kg BW) were found to have improved hematological indices but also exhibited large iron deposits in hepatic macrophages as well as fully saturated levels of ferritin in the liver which could possibly indicate the onset of iron overload.

Parenteral iron toxicity has been noted for piglets born from sows fed inadequate levels of vitamin E and selenium (Velásquez and Aranzazu, 2004). Piglets from these

sows showed signs of toxicity (hypothermia, anorexia, and oliguria) as early as 6 hours after administration of 200 mg iron dextran which led to an 80 % mortality incidence.

Patterson et al. (1969) demonstrated that an iron dextrose injection (47 mg iron/ kg BW) administered to piglets that were vitamin E deficient resulted in an increase in muscle peroxides that were twice the levels of those observed before the injection, indicating that the iron injection initiated degenerative myopathy through peroxidative damage to muscle. It was also suggested that iron toxicity in piglets is exacerbated by a vitamin E deficient state as skeletal muscle has an increased affinity for iron. Notably, the experiment by Patterson et al. (1969) used iron dextrose rather than iron dextran, which iron dextrose was observed to be absorbed more rapidly than iron dextran and there is a reduced risk of iron toxicity when iron dextran is administered as iron is tightly bound to the larger polysaccharide molecule. Later work by Patterson et al. (1971) resulted in similar findings to previous work as degenerative myopathy was observed through stainable iron deposits (Prussian blue method) in the cytoplasm and nuclear membrane of affected muscle fibers as well as reticuloendothelial cells.

Kadis et al. (1984) showed that piglets challenged with Escherichia coli (E. coli) after receiving an iron injection (200 mg iron dextran) either 30 minutes or 2 days after birth resulted in a numerical increase in mortality compared to piglets that were not challenged with E. coli but administered iron injections. The higher mortality that was observed was likely due to the greater incidence of diarrhea that was observed with iron injected pigs challenged with E. coli. Altogether, iron is cleared from the circulating serum and deposited in storage sites rather quickly. However, in situations of vitamin E or selenium deficiency, high levels of iron may be detrimental to pigs and cause iron overload.

2.8.5.2 Hepcidin

Hepcidin is a key negative regulator in iron metabolism that is found in liver hepatocytes (Ganz, 2003). It aids in iron homeostasis by inhibiting iron influx into the plasma from the duodenal enterocytes, macrophages that recycle iron, and hepatocytes that store iron by binding and degrading ferroportin (Nemeth et al., 2004b; Nemeth and Ganz, 2006). Also by controlling the expression of ferroportin on the basolateral membrane, hepcidin can control the rate of iron absorption (Munoz et al., 2009). Hepcidin production is elevated by iron and inflammation whereas it is reduced in anemic and hypoxic states (Nemeth and Ganz, 2006). Rivera et al. (2005) showed the regulating effect of hepcidin by injecting mice with a synthetic form of hepcidin and finding that as early as 1-hour post-injection there was a significant decrease in serum iron.

2.8.6 Immune function

Iron is a crucial element for its role in immune development by promoting growth for immune cells and its close relation with cell-mediated immune responses and cytokines (Wessling-Resnick, 2010; Pu et al., 2018). Anemia of inflammation or hypoferremia is a decrease of iron in the serum and is associated with obstruction of systemic iron homeostasis caused by the hepcidin antimicrobial peptide (HEPC) (Nemeth et al., 2004a; Roy et al., 2007). Hepcidin synthesis is greatly increased under infectious and inflammatory conditions, in turn decreasing iron absorption and becoming the key factor to anemia of inflammation (Nemeth and Ganz, 2006). Hepcidin has antimicrobial properties but its ability to decrease circulating iron which is a growth factor for invading pathogens is thought to be more beneficial to the body (Pagani et al., 2011). Work done

in mice that were in an iron-deficient state where hepcidin levels are low, had a higher inflammatory response to LPS (Pagani et al., 2011).

2.9 Nutrient interactions

2.9.1 Non-competitive and competitive inhibition

Dietary interactions between minerals often occur in the alimentary canal. It is widely accepted that inorganic substances with similar chemical properties will interact greatly with each other because of the shared use of absorption channels resulting in competition or coadaptation (Hill and Matrone, 1970; Rosenberg and Solomons, 1982). Work by Gunshin et al. (1997) showed a wide range of metal elements (Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+}) that DMT1 can transport.

It is thought that zinc transporter protein 14 (ZIP14) plays a role in iron transport in hepatocytes and acinar cells under pathological conditions by moving non-transferrin-bound-iron (NTBI) when there is an overload of iron (Camaschella and Pagani, 2018). It is well thought that when zinc and iron are present in ionic form, there will be a competitive interaction between the two (Solomons, 1988). The competitive interaction between iron and zinc was demonstrated early using a zinc tolerance test which resulted in a decrease in circulating zinc when the ratio of iron:zinc increased from 1:1 to 3:1 (Solomons and Jacob, 1981).

In addition to zinc, copper has been known to be another mineral that interacts with the absorption of iron. Copper has very similar properties to those of iron. Copper is also a key component for hemoglobin as it stimulates the maturation of red blood cells and increases their survival time (Lloyd et al., 1960). More specific, copper in the form of ceruloplasmin in circulating plasma causes ferroxidase activity to release hepatic iron for

hemoglobin synthesis in erythropoiesis (Miller, 1991). Ceruloplasmin is a metalloprotein that contains eight copper atoms per molecule and is the main source of copper in the circulating plasma. Hill et al. (1983) created copper-deficient piglets from sows fed high levels of zinc (5000 mg/kg Zn as zinc oxide). These pigs were fed supplemental copper diets (0, 5, or 10 mg/kg Cu as copper sulfate) at 3 to 5 days after birth and the pigs that were fed 0 mg/kg Cu showed signs of anemia by 35 days of age with low hemoglobin and ceruloplasmin concentrations.

Lee et al. (1968) demonstrated that administering an intramuscular injection of iron to copper-deficient piglets did not prevent hypochromic and microcytic anemia. It was also observed that there were iron metabolism defects in the duodenal mucosa, reticuloendothelial system (RES), and hepatic parenchymal cells that led to an impairment in the release and transfer of iron. However, later work by Ragan et al. (1969) showed that the defects seen in iron metabolism associated with copper deficiency in pigs can be reversed with an intravenous injection of ceruloplasmin. This leads to the hypothesis that copper deficiency causes hypoceruloplasminemia resulting in an “iron block”.

Astrup and Lyso (1986) demonstrated a reduction in hepatic copper levels compared to control values when dietary iron was increased at levels of 20 and 40 times that of dietary copper. Work by Klevay (2001) using rats fed low or high levels of copper in addition to high levels of iron suggest that the copper requirement is increased when the iron level is increased due to the low cardiac and hepatic copper concentration observed for the low but not high copper fed rats. It has also been demonstrated that hepatic copper levels in rodents are greater when an iron-deficient state is induced (Sourkes et al., 1968;

Owen, 1973). Although it is not completely understood but apparent that there are interactions between iron and copper absorption, Collins et al. (2010) suggests that the interactions are physiological responses for managing overall body metal levels. Calcium can also act as a non-competitive inhibitor to iron on the interaction with DMT1, reducing dietary iron absorption (Shawki and Mackenzie, 2010). Other dietary constituents that could have an impact on iron absorption are phytates, carbonates, phosphates, oxalates, and tannates which can cause ferric iron to precipitate and form macromolecules rendering it unavailable for absorption (Conrad and Umbreit, 2000).

2.9.2 Facilitators of iron absorption

Due to the favorable redox capacity of iron, it is thought that it is better absorbed when facilitated by other nutrients. To remain in the reduced state for absorption, ferrous iron must rely on continuous reduction or chelation that prohibits exposure to oxygen. Ascorbic acid is the best known reducing agent in the diet (Conrad and Schade, 1968; Solomons, 1988). Ascorbic acid consumed in the diet helps maintain the solubility of iron by keeping it in the reduced state (Hunt, 2005). Work by Fidler et al. (2004) demonstrated that the addition of erythorbic acid (a stereoisomer of ascorbic acid) to the diet at ratios 2:1 and 4:1 of iron, increased iron absorption in women by 10 and 18%.

2.10 Conclusion

Piglet health status and growth performance are critical during the pre and postweaning periods as it is the foundation for success in subsequent periods of life. A major contributor to this is iron status. Iron is a vital mineral that is needed to transport oxygen throughout the body, as well as many other cellular and enzymatic functions that are required for living organisms. Without an exogenous supply of iron given shortly

after birth, piglets become iron deficient and anemic. Even when iron is supplemented to newborn piglets, there is still a chance that iron deficiency may occur depending on the growth rate of the pig. In contrast, when the iron status is improved in pigs before weaning, there is a greater chance for an enhanced postweaning performance which leads to heavier pigs and fewer days to market weight. In the current swine industry, the incidence of iron deficiency at weaning has recently been estimated to cost producers millions of dollars (Olsen, 2019). However, the iron deficiency issue can potentially be corrected by simply supplying more iron. To precisely correct the iron issue, additional research is needed to truly understand the time course of iron status in piglets during lactation, weaning, and postweaning.

Therefore, the objective of the present research was to assess and evaluate the iron status of piglets after receiving an iron injection after birth (Chapter 3 and 4), as well as determine the effects of an additional iron injection administered before weaning on growth performance and iron status (Chapter 5).

CHAPTER 3. Assessment of the iron status of young pigs in a confinement herd

3.1 Abstract

The objective of the present experiment was to assess the iron status of the University of Kentucky swine herd by evaluating the relationship between BW and hematological status at various time points for young pigs. A total of 120 crossbred pigs (1 d of age; initial BW of 1.77 ± 0.38 kg) were selected for evaluation of iron status from 1 d of age to 35d-postweaning. Body weight and blood samples were collected at d 1, weaning (d 18 to 25), 21d-postweaning, and 35d-postweaning. All pigs were administered an intramuscular injection of iron dextran providing 150 mg iron on d 1 of age after the blood sample was collected. Pigs were weaned to nursery pens at an age of 18 to 25 d where they received a common two-phase nursery diet. The nursery diet was formulated to meet or exceed the NRC (1998) nutrient requirement estimates for energy and protein for pigs 7 to 25 kg. Blood samples that were collected were analyzed for a complete blood count (CBC). At weaning 50% of the pigs were below the industry hemoglobin concentration (Hb) critical limit of 11 g/dL. In addition, at weaning there was a negative relationship between hemoglobin concentration and BW or BW gain; indicated by the decrease in Hb concentration as BW and BW gain increased (Hb =-0.489(BW) and Hb = -0.659(BW gain); $P < 0.0001$). Following weaning, at 21d-postweaning and 35d-postweaning there were only 2 pigs (2%) considered below the hemoglobin critical limit. The relationship of hemoglobin concentration and BW at 21d-postweaning became positive (Hb = 0.028 (BW); $P = 0.3481$) and this was even more pronounced at 35d-postweaning where the relationship of Hb concentration and BW was more positive (Hb = 0.101(BW); $P < 0.0001$). The results of this experiment indicate that at weaning there

are pigs in the UK swine herd that are below the critical limit of hemoglobin which may indicate iron deficiency, however by 21d- postweaning and 35d-postweaning the incidence is reduced greatly indicating that the iron requirement is being met by the nursery diet. Therefore, the UK herd is suitable for the evaluation of the time course of iron deficient anemia and the potential means to reduce or eliminate its adverse effects.

Key Words: anemia, iron deficiency, iron, pigs, weaning

3.2 Introduction

Pigs reared in confinement production systems have very limited sources of iron. The sow is responsible for the provision of nutrients to her developing offspring which can often be a hard task given that swine are litter bearing species. Consequently, piglets are born with very low hepatic iron reserves and, to exacerbate the issue, the primary feed source for pigs' post-partum is sow milk which has been demonstrated to be very low in iron (Venn et al., 1947).

For these reasons, it is a routine practice in swine production to administer a supplemental iron source shortly after birth. Over the years the supplemental supply of iron has often been in the form of iron dextran, as an intramuscular injection. Iron injections generally range from 100-200 mg iron and are administered anywhere from the time of birth to 1 week following birth (Szudzik et al., 2018). This early iron injection has been used for numerous years to give piglets the iron supply they need until they are weaned to a diet which will contain more iron than is contained in the milk.

However, with modern genetics, elevated productivity levels, and rapid growth performances there have been concerns regarding the adequacy of the early-life iron injection. Recent research has demonstrated that heavier and fast-growing piglets may outgrow the initial iron supplement as they are susceptible to have a lower iron status at weaning (Bhattarai and Nielsen, 2015; Jolliff and Mahan, 2011). Therefore, the objective of the present experiment was to assess the iron status of the UK swine herd by evaluating the relationship between BW and hematological status at various time points for young pigs.

3.3 Experimental procedures

This experiment was conducted in environmentally controlled rooms at the University of Kentucky Swine Research Center under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

3.3.1 Animals, housing, management, and experimental design

A total of 120 newborn, crossbred piglets from 13 sows [52 barrows, 68 gilts; (Yorkshire × Landrace× Duroc)] with a mean initial BW of 1.77 ± 0.38 kg were selected for assessment of iron status through the lactation and nursery periods. All piglets were weighed and injected with a single intramuscular (IM) injection of iron dextran (Henry Schein Animal Health, Dublin, OH), containing approximately 150 mg iron one d after birth. At this time, all pigs underwent tail docking, needle teeth removal, and ear notching procedures. There was no cross-fostering during this experiment.

Pigs on the experiment were kept in farrowing crates with their respective dams. Farrowing crates (1.52 × 2.13 m^2) were in environmentally-controlled rooms equipped with a plastic-coated, woven wire floor, heat lamps, and nipple waterers for the sow and piglets. Sows had ad libitum feed access through a feed trough on the front gate of the farrowing crate. All animals had unlimited access to water, piglets were not offered creep feed but were not restricted from the sow feed.

Piglets were weaned to a nursery site at 18 to 25 days of age. In the nursery, pigs were placed in elevated pens (1.22 m × 1.22 m) with plastic coated, welded wire flooring. Pigs were placed in pens based on BW, considering that stocking density is a contributor

to growth performance. Pens consisted of a three-hole plastic feeder and a nipple waterer from which all pigs had ad libitum access during the 35 d nursery period.

3.3.2 Experimental diets

The diets fed in the nursery were formulated to meet or exceed the NRC (1998) requirement estimates for energy and protein for pigs based on body weight. All pigs were assigned to the same diet for the two nursery phases. Phase I and II diets (Table 3.1) were fed for the first 21 days after weaning and from 21d-postweaning to 35d-postweaning, respectively.

Table 3 1 Composition of nursery diets (as-fed basis)

Item	Phase I	Phase II
Ingredient, %		
Corn	46.28	59.16
Soybean meal, 48% CP	15.95	19.85
Grease, choice white	2.60	1.95
Fish meal (Menhaden)	7.00	4.00
Spray-dried animal plasma	4.00	0.00
Whey dried	22.50	12.50
L-Lysine•HCl	0.18	0.30
DL-Methionine	0.11	0.12
L-Threonine	0.06	0.09
Dicalcium phosphate	0.25	1.15
Limestone	0.33	0.50
Salt	0.30	0.21
Trace mineral premix[1]	0.05	0.05
Vitamin premix[2]	0.08	0.08
Choline chloride	0.05	0.05
Zinc oxide	0.20	0.00
Copper sulfate	0.07	0.00
Total	100.00	100.00
Calculated composition		
Metabolizable energy, kcal/kg	3371.00	3373.00
Crude protein, %	21.94	21.94
SID Lysine, %	1.54	1.54
Calcium, %	0.79	0.79
STTD Phosphorus, %	0.72	0.72

[1]Supplied the following per kilogram of diets: 16.67 mg of Mn as manganous sulfate, 33.33 mg of Fe as ferrous sulfate, 41.67 mg of Zn as zinc sulfate, 6.67 mg of Cu as copper sulfate, 0.11 mg of I as calcium iodate, and 0.10 mg of Se as sodium selenite.

[2]Supplied the following per kilogram of diets: 8,490 IU of vitamin A; 2,124 IU of vitamin D3; 56.52 IU of vitamin E; 6.30 IU of vitamin K; 0.024 mg of vitamin B12; 0.208 mg of biotin; 18.91 mg of pantothenic acid; 0.152 mg of folic acid; 37.61 mg of niacin; 3.77 mg of vitamin B6; and 1.04 mg of thiamin.

3.3.3 Data and sample collection

3.3.3.1 Growth performance response measures

Pig body weights were recorded at birth, weaning, 21d-postweaning, and 35d-postweaning. During the time in the nursery, feeders were checked daily to ensure proper flow and amount of feed present. Water nipple heights were adjusted to ensure easy access based on the size of the pigs.

3.3.3.2 Blood collection

All blood samples were collected by jugular venipuncture. Samples were collected from all pigs at birth, weaning, 21d-postweaning, and 35d-postweaning. Whole blood was collected by a 10 mL syringe with a 1", 18 gauge needle and transferred into EDTA (purple-top) tubes (BD Vacutainer®, Franklin Lakes, NJ). Samples were placed on ice and transported to the University of Kentucky Veterinary Diagnostic Lab (UKVDL) within 4 hours of collection for complete blood count (CBC) analysis.

3.3.4 Sample processing and laboratory analysis

3.3.4.1 Blood analysis

CBC analysis was performed for all pigs at all collection times during this experiment by the UKVDL. The UKVDL analyzed the whole blood samples for a CBC using a veterinary hematological analyzer (Forcyte Veterinary Hematology Analyzer, Oxford Science, Oxford, CT). Before analysis, all blood samples were thoroughly mixed and brought to room temperature. The CBC analysis consisted of hemoglobin concentration (Hb), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

3.3.5 Statistical analysis

All data were subjected to a statistical outlier test through Grubb's test outlier calculator (GraphPad Software, San Diego, CA, USA). For all data, ranges and means were calculated. Selected data underwent scatter plot analysis with the trendline, graphical equation, and the R^2 being calculated.

3.4 Results

A total of 120 crossbred pigs were weighed and bled the d after birth, weaning, 21d-postweaning, and 35d-postweaning. Two blood samples clotted at both birth and 35d-postweaning and were unable to be analyzed for CBC.

Table 3.2 provides the ranges and means of BW and CBC measures throughout the experiment. During the duration of the study, mean Hb, HCT, and RBC all increased as time increased. At birth and weaning, the mean hemoglobin concentration is below the optimal hemoglobin classification (11 g/dL), subsequently, at 21 and 35 days postweaning the mean hemoglobin concentration surpassed the optimal hemoglobin concentration. At weaning, 50 % of the 120 pigs were classified with hemoglobin concentrations below the optimal concentration (Table 3.3). But by 21 and 35 days postweaning, only 2% of pigs remained below that critical limit.

Figure 3.1 represents the hemoglobin concentration of pigs based on their birth weight. At birth there is a large portion of the population that is below the optimal hemoglobin limit (80%); however, there is a positive relationship (Hb = 1.528(BW)) of hemoglobin concentration and body weight at birth.

Later, at weaning (Figure 3.2) the slope is negative (Hb = -0.489(BW)) demonstrating a decrease in hemoglobin concentration as weaning BW increases. Also at weaning, there is a more negative relationship (Hb = -0.659(BW gain)) of hemoglobin concentration and total BW gain to weaning (Figure 3.3). Following weaning, there are only two pigs that are below the optimal hemoglobin limit. In Figure 3.4, the relationship between hemoglobin concentration and 21d-postweaning BW shows the trendline becoming positive (Hb = 0.028(BW)). Furthermore at 35 days postweaning the trendline becomes even more positive (Hb = 0.101(BW)) (Figure 3.5).

Table 3 2 Ranges and means of body weights and CBC at different time points[1,2,3]

Variable	Unit	Birth	Weaning	21d-Postweaning	35d-Postweaning
BW	kg				
Minimum		0.96	3.10	8.30	14.91
Maximum		2.76	9.82	22.59	32.14
Mean		1.77	6.21	14.41	23.82
Hb	g/dL				
Minimum		3.7	8.6	10.9	10.6
Maximum		13.0	15.1	14.4	14.6
Mean		9.5	10.9	12.7	12.8
HCT	%				
Minimum		10.6	25.1	27.9	26.4
Maximum		38.6	43.4	37.2	41.8
Mean		28.0	31.9	32.2	34.0
RBC	10^6/µL				
Minimum		1.74	4.18	5.18	5.08
Maximum		6.54	7.83	7.48	5.55
Mean		4.68	5.65	6.08	6.18
WBC	10^3/µL				
Minimum		3.24	3.54	5.58	6.40
Maximum		18.66	20.58	39.72	22.44
Mean		7.68	7.34	12.45	12.83
MCV	fL				
Minimum		51.3	47.4	46.2	47.2
Maximum		69.1	65.4	59.1	60.5
Mean		59.9	56.5	53.1	55.0
MCH	pg				
Minimum		16.9	16.2	12.4	18.1
Maximum		50.6	22.4	23.5	23.1
Mean		20.6	19.4	20.8	20.8
MCHC	g/dL				
Minimum		30.7	19.5	36.4	33.0
Maximum		37.8	37.6	41.9	40.9
Mean		34.0	34.3	39.3	37.8

[1]CBC data at birth and 35d-postweaning uses 118 pigs, CBC data for weaning and 21d-postweaning uses 120 pigs, BW data for all time points uses 120 pigs.
[2]CBC measures include hemoglobin concentration (Hb), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).
[3]Weaning was at 18 to 25 days of age.

Table 3 3 Absolute and percentage (%) of pigs in hemoglobin categories at different time points[1]

		Hemoglobin concentration, g/dL		
		Optimal	Sub-clinical deficiency	Clinical deficiency
Variable	n	> 11	11 to 9	< 9
Birth	118	24 (20)	52 (44)	42 (36)
Weaning	120	60 (50)	58 (48)	2 (2)
21d-Postweaning	120	118 (98)	2 (2)	0 (0)
35d-Postweaning	119	117 (98)	2 (2)	0 (0)

[1]Weaning was at 18 to 25 days.

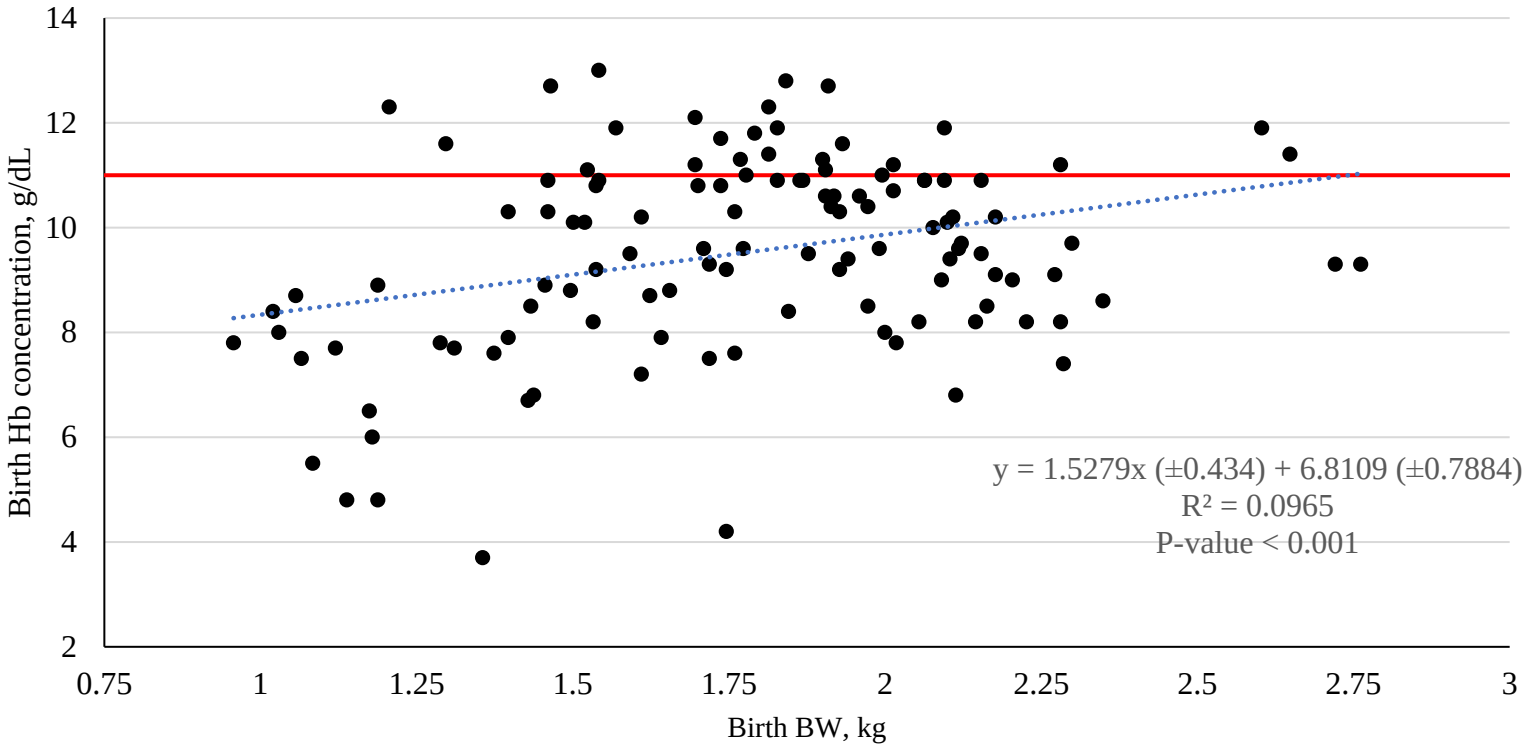

Figure 3.1. Relationship of Hb concentration and BW at birth (n = 118). The dashed line represents the linear trendline and the solid horizontal red line is fixed on the y-axis at 11 g/dL to represent the critical limit of hemoglobin concentration.

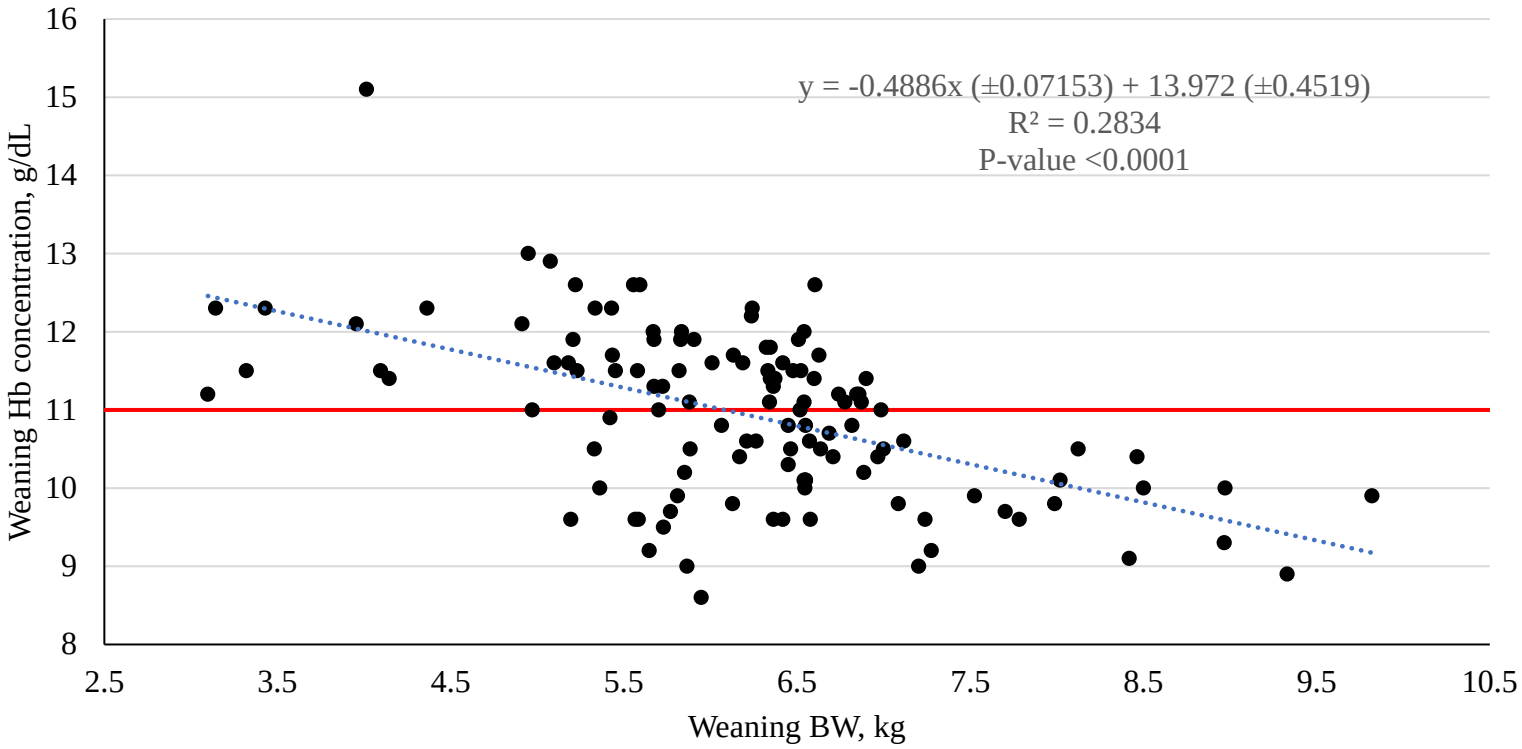

Figure 3.2. Relationship of Hb concentration and BW at weaning (n = 120). The dashed line represents the linear trendline and the solid horizontal red line is fixed on the y-axis at 11 g/dL to represent the critical limit of hemoglobin concentration.

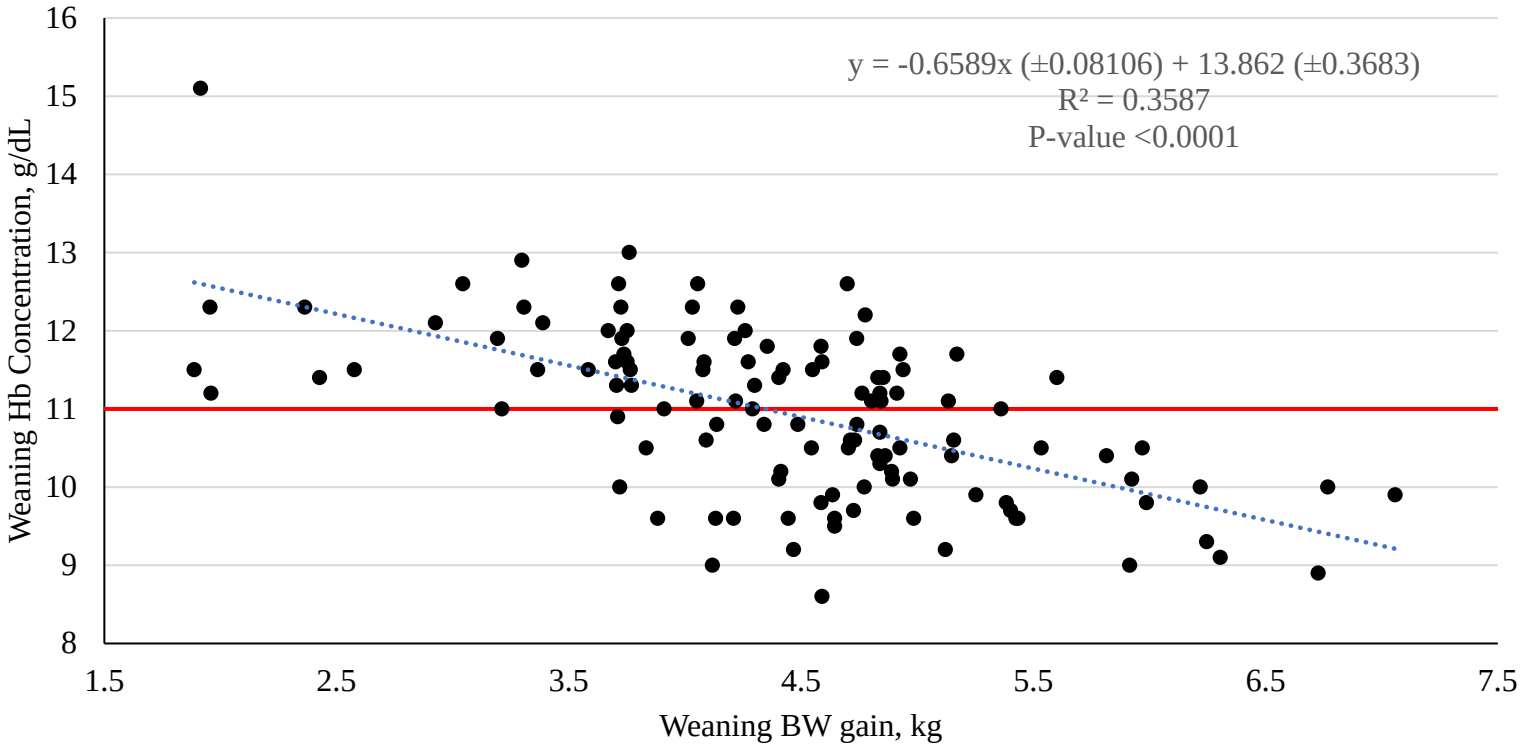

Figure 3.3. Relationship of Hb concentration and BW gain at weaning (n = 120). The dashed line represents the linear trendline and the solid horizontal red line is fixed on the y-axis at 11 g/dL to represent the critical limit of hemoglobin concentration.

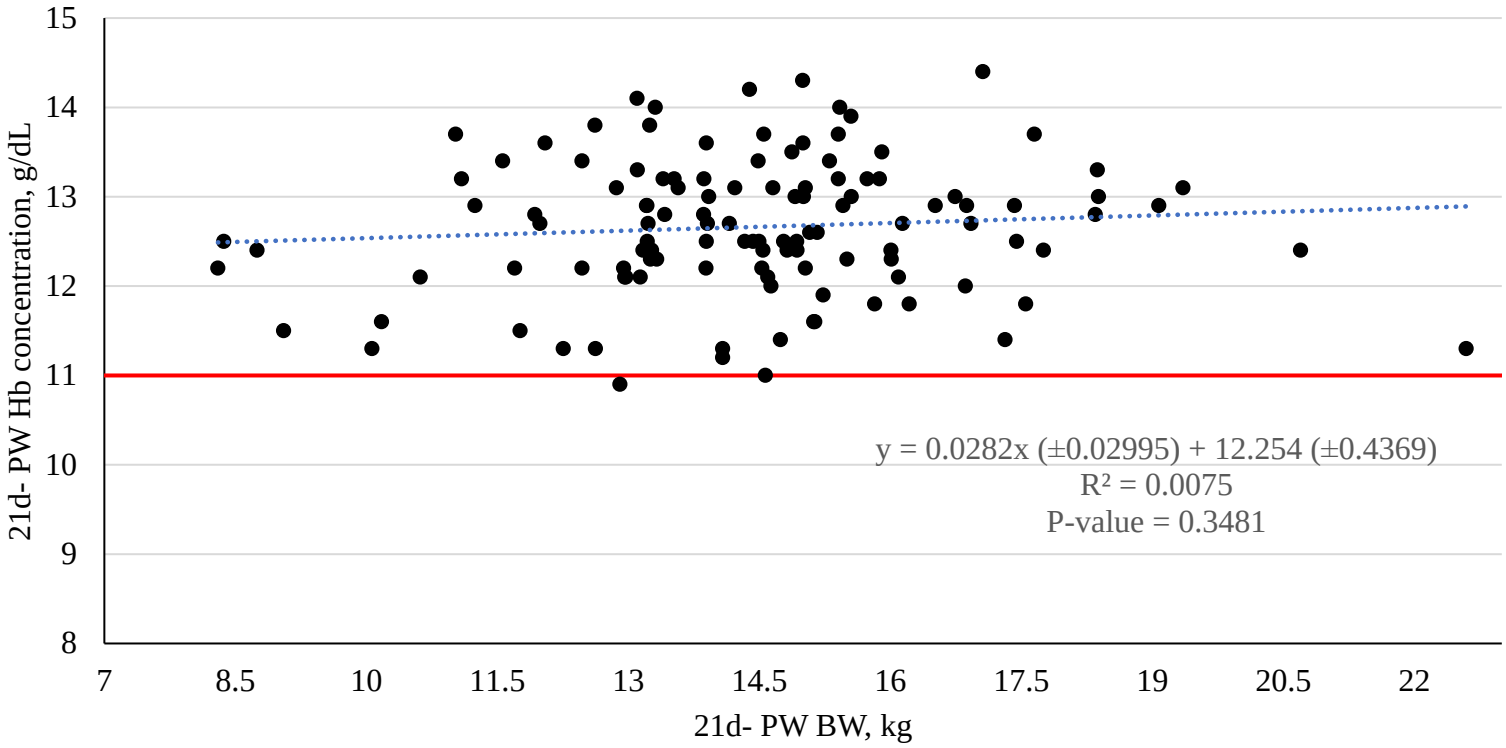

Figure 3.4. Relationship of Hb concentration and BW at 21d-postweaning (PW) (n = 120). The dashed line represents the linear trendline and the solid horizontal red line is fixed on the y-axis at 11 g/dL to represent the critical limit of hemoglobin concentration.

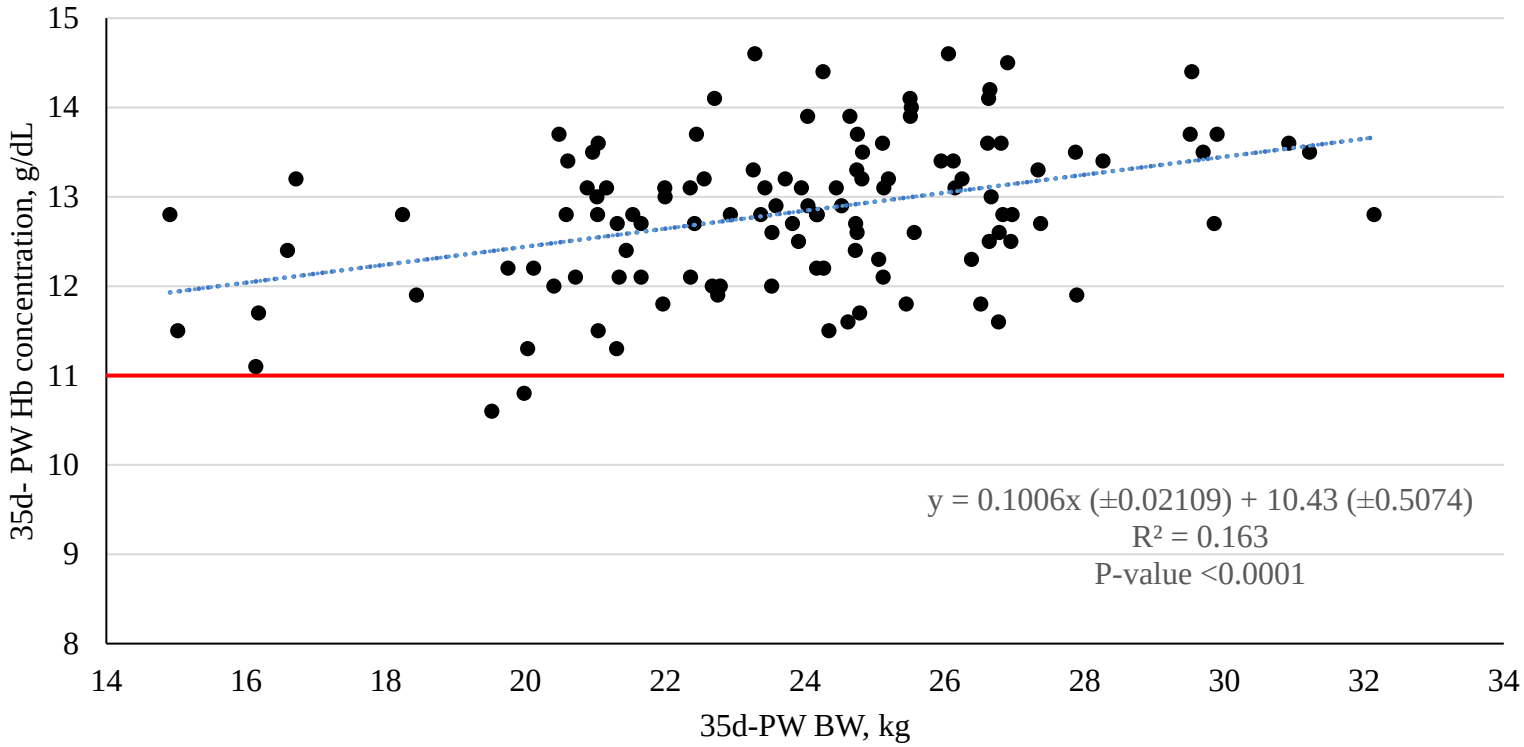

Figure 3.5. Relationship of Hb concentration and BW at 35d-postweaning (PW) (n = 119). The dashed line represents the linear trendline and the solid horizontal red line is fixed on the y-axis at 11 g/dL to represent the critical limit of hemoglobin concentration.

3.5 Discussion

At birth of the current experiment, there was a large group (80%) of piglets with hemoglobin levels below 11 g/dL. In the developing fetus, iron is transported from the mother to the fetus through endometrial secretions of uteroferrin across the maternoplacental barrier which is limited in the pig (Roberts and Bazer, 1980). The results of the current experiment confirm earlier research, where Venn et al. (1947) demonstrated that piglets are born with a low body iron. With the exception of low birth weight humans, pigs are the only mammalian species that experience neonatal iron deficiency which is largely attributable to low iron reserves at birth. However, there is limited work on the course of blood measures in young pigs from birth to weaning.

In the current experiment, 50% of the pigs were found to be below the optimal hemoglobin concentration (< 11 g/dL) at weaning, which is an improvement from d 1. This could be attributable to the iron injection administered after the blood sample was collected on d 1. However, there is a large percentage of piglets at weaning with below optimal hemoglobin levels indicating that the iron supplement may not be adequate to sustain all pigs until weaning. More so, recent work has also shown populations of pigs below the optimal hemoglobin limit at weaning (Bhattarai and Nielsen, 2013; Jolliff and Mahan, 2011; Perri et al., 2016). Jolliff and Mahan (2011) also showed that the pigs below the optimal hemoglobin concentration at weaning were more likely to be the faster-growing pigs rather than small or slow-growing pigs. This is in agreement with the current experiment, where at weaning there was a negative relationship between hemoglobin concentration and BW or BW gain (Figures 3.2 and 3.3).

At 21d- and 35d-postweaning there were only 2 pigs (2%) that were considered sub-clinical iron deficient and no pigs considered anemic. The 2 pigs that were below optimal hemoglobin concentrations were a result of low iron status at weaning and poor feed intake that was estimated from the daily gain in the nursery. Together these pigs had BW gains that were 30 g and 200 g less than the average at 21d- and 35d-postweaning respectively. The low occurrence of postweaning iron deficiency in the current experiment is contrary to results from Perri et al. (2016), who reported a greater incidence of iron deficiency and anemia 3 weeks postweaning compared to the incidence at weaning. Notably, the current experimental diet only contained 0.2% Zinc oxide, which is a much lower level of zinc (20 mg/kg ZnO) than diets used in the study of Perri et al. (2016) (250-7000 mg/kg ZnO). Thus because of the competitive effect that zinc has with iron for transport by DMT-1 (Gunshin et al., 1997), the higher incidence of iron deficiency and anemia observed by Perri et al. (2016) can plausibly be explained.

3.6 Conclusion

In the current experiment, piglets had low hemoglobin concentration at birth, and after receiving an iron injection containing 150 mg iron at d 1, there was still a large portion (50%) of the pigs considered below optimal Hb concentration (11 g/dL) at weaning (d 18-25). Additionally, there was a negative relationship between weaning hemoglobin concentration and both weaning BW/ BW gain. In contrast, 21d- and 35d-postweaning hemoglobin concentration had a positive relationship with BW. Furthermore in the nursery, there were only 2 pigs (2%) that were considered iron-deficient compared to the 50% observed at weaning. Further research to focus on the time course of the iron status of the pig through the lactation and nursery periods may provide more precise

information of when the iron supply runs low in piglets and suggest when it should be addressed with some type of intervention.

CHAPTER 4. Effects of increasing iron dosage to newborn piglets on growth performance, hematological measures, and tissue mineral concentrations pre and postweaning

4.1 Abstract

The objective of the current experiment was to evaluate and determine the course of the blood profile, growth performance, and tissue mineral concentration of pigs during pre and postweaning periods after receiving an iron injection at birth. In a 52-d trial, a total of 70 piglets (initial BW of 1.51 ± 0.56 kg) from 7 litters were assigned to 1 of 5 different iron injection dosage treatments on d 0. Injectable iron dextran treatments were as follows: 0, 50, 100, 200, and 300 mg iron. Pigs were weaned to nursery pens at d 22 where they were housed by treatment and fed a common nursery diet. BW was measured on d 0 (before injection), 1, 2, 3, 4, 6, 8, 11, 14, 17, 22 (weaning), 23, 24, 25, 29, 38, 44, and 52. Blood was collected at the same time points as listed above with the exception of d 44. Tissue samples were also collected on d 22, 38, and 52. The individual pig served as the experimental unit and CBC data was analyzed as repeated measures. Overall, the pigs that were not injected with iron had the lowest growth performance, complete blood count (CBC), and tissue mineral measures that indicated a state of anemia. During lactation, at Weeks 1 and 3 there was a linear increase ($P < 0.05$) in ADG for increasing injectable iron dosages. After weaning, at Weeks 4 and 5 there were both linear improvements ($P < 0.01$) for ADG in response to iron dosage, additionally at Week 5 there was a quadratic tendency ($P = 0.07$) for greater ADG with the 300 mg iron dose having the greatest ADG. Increasing iron dosage also resulted in a quadratic increase ($P = 0.03$) in ADFI for the overall nursery period (d 22 to d 52). Hemoglobin (Hb)

concentration improved (P = 0.01) with increasing injectable iron as early as d 1 and continued to d 38, thereafter (d 52) no differences in Hb concentration were observed. A similar pattern where an increase was observed early on in the experiment and continued to d 38 but then disappeared at d 52 existed with the other CBC measures. The iron concentration of all tissues (liver, spleen, heart, and kidneys) were greater ($P \leq 0.01$) at weaning with increasing iron dosage. Interestingly, at weaning and d 38, the absolute and relative heart weight was higher ($P \leq 0.02$) for pigs receiving no iron injection. Results indicate that an iron injection administered shortly after birth is vital for proper growth and hematological functions. Additionally the administration of 300 mg iron injection had no negative effects, and may also provide a more consistent level of iron during the pre and postweaning periods for pigs.

Key Words: anemia, iron, iron injection, dosage, piglets

4.2 Introduction

Current advancements with genetics in the swine industry have led to an increase in growth rates of newly born piglets (Pig Champ, 2019), suggesting a greater demand for certain nutrients. Due to the increases in body size, low amounts of iron in sow milk, and small initial iron reserve, it is a common practice to provide pigs with an intramuscular injection of iron shortly after birth in order to prevent anemia. However, current research has questioned if the single iron injection early in life is sufficient for the lactation and weaning periods before the pig starts to consume nursery diets (Jolliff and Mahan, 2011; Perri et al., 2016). More recent work by Morales et al. (2018) demonstrated that after administration of an iron injection (200 mg iron) at processing, serum iron was declining past initial serum iron concentrations by days 14 to 17. These findings could be related to the common occurrence of newly weaned pigs becoming lethargic leading to poor nursery performance.

It is evident that optimizing iron status at weaning can have benefits to the pig in the subsequent nursery period. However, there is limited information on the time course of iron status of the pig from the time of the initial iron injection to weaning. By understanding the natural decline of iron status before weaning, more targeted interventions for maximizing hematological status and growth performance in pigs can be made. Therefore, the objective of the present experiment was to critically monitor the time course of the blood profile, growth performance, and tissue mineral concentration of pigs during pre and postweaning periods after receiving various amounts of iron in an iron injection at birth.

4.3 Experimental procedures

This experiment was conducted in environmentally controlled rooms at the University of Kentucky Swine Research Center under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky

4.3.1 Animals, housing, management, and experimental design

A total of 70, one -day-old pigs [32 barrows, 38 gilts; (Yorkshire x Landrace) x Large White] from 7 litters with an initial BW of 1.51 ± 0.26 kg were used in this experiment. Piglets were weighed and randomly allotted within litters shortly after birth to 5 different iron dextran injection treatments (14 pigs/treatment). The five different treatments consisted of a single intramuscular (IM) injection of 0, 50, 100, 200, or 300 mg iron from iron dextran (Henry Schein Animal Health, Dublin, OH). Before treatments were administered, all piglets underwent normal farm processing procedures (removal of needle teeth, tail-docking, and ear-notching). All male pigs were castrated on d 8 of the experiment and no cross-fostering occurred. All iron injections were administered in the right neck muscle of the piglets. Early during the experiment (d 4), 1 pig from the 200 mg iron treatment died, resulting in individual BW and ADG means represented by 13 pigs per treatment for that treatment.

Pigs on the experiment were kept in farrowing crates with their respective dam. Farrowing crates (1.52 × 2.13 m^2) were in environmentally-controlled rooms equipped with a plastic-coated, woven wire floor, heat lamps, and nipple waterers for sows and piglets. Sows had ad libitum access to feed through a feed trough on the front gate of the farrowing crate. All animals had unlimited access to water, piglets were not offered creep

feed but were not restricted from the sow feed. The experiment was carried out for a total of 52 days.

Pigs were weaned to a nursery site at 22 days of age. In the nursery, pigs were allotted to pens based on BW and treatment. Pens consisted of 4 or 5 pigs per pen and were equalized between treatments to 3 pens per treatment.

All nursery pens (1.22 m × 1.22 m) were elevated off of the ground in an environmentally-controlled room with plastic-coated wire flooring. All pens were equipped with a three-hole plastic feeder and a nipple waterer. Pigs had ad libitum access to water and feed for the duration of the experiment. Pigs from all treatments were fed common two-phase nursery diets.

4.3.2 Experimental diets

All pigs received a common two-phase nursery diet sequence that was formulated to meet or exceed NRC (2012) requirement estimates for pigs 7 to 25 kg (Table 3.1). Phase I was fed for the first 16 days of the nursery (d 22 to 38) while Phase II was fed from d 38 to 52. The trace mineral premix used in both phases supplied the following per kilogram of the diet: 50 mg of Mn as manganous sulfate, 100 mg of iron as ferrous sulfate, 100 mg of Zn as zinc sulfate, 18 mg of Cu as copper sulfate, 0.7 mg of I as calcium iodate, and 0.30 mg of Se as sodium selenite.

Phase I and II diets were analyzed for selected trace mineral concentrations (Fe, Zn, and Cu). The analyzed mineral composition of Phase I diets were 117 ppm Fe, 118 ppm Zn, and 17 ppm Cu. For Phase II diets, the analyzed mineral composition was 220 ppm Fe, 108 ppm Zn, and 15 ppm Cu. Both diets were also analyzed for Ca and P content, for

Phase I the Ca and P concentration was 0.76% and 0.61%, respectively. For the Phase II diets Ca and P were 0.70% and 0.64%, respectively.

Table 4.1. Composition of sow lactation and piglet nursery diets (as-fed basis)

Item	Sow lactation	Phase I	Phase II
Ingredient, %			
Corn	69.57	50.55	57.46
Soybean meal, 48% CP	27.00	28.50	32.50
Grease, choice white	-	2.00	2.00
Fish meal (Menhaden)	-	5.00	0.00
Spray-dried animal plasma	-	2.00	0.00
Whey dried	-	10.00	5.00
L-Lysine•HCl	0.04	0.07	0.24
DL-Methionine	-	0.05	0.13
L-Threonine	-	0.07	0.14
Dicalcium phosphate	1.60	0.33	0.97
Limestone	0.90	0.77	0.90
Salt	0.50	0.50	0.50
Trace mineral premix[1]	0.10	0.10	0.10
Vitamin premix[2]	0.10	0.04	0.04
Santoquin[3]	0.02	0.02	0.02
Other[4]	0.17	-	-
Total	100.00	100.00	100.00
Calculated Composition			
Metabolizable energy, kcal/kg	3298.00	3423.00	3404.00
Crude protein, %	18.66	23.79	21.22
SID Lysine, %	0.87	1.35	1.23
Calcium, %	0.84	0.80	0.70
STTD Phosphorus, %	0.40	0.36	0.29

[1]Supplied the following per kilogram of diets: 50 mg of Mn as manganous sulfate, 100 mg of Fe as ferrous sulfate, 125 mg of Zn as zinc sulfate, 18 mg of Cu as copper sulfate, 0.35 mg of I as calcium iodate, and 0.30 mg of Se as sodium selenite.

[2]Supplied the following per kilogram of nursery diets: 4,245 IU of vitamin A; 1,062 IU of vitamin D3; 28.3 IU of vitamin E; 3.2 IU of vitamin K; 0.012 mg of vitamin B12; 9.45 mg of pantothenic acid; 0.104 mg of biotin; 0.076 mg of folic acid; 18.81 mg of niacin; 1.89 mg of vitamin B6; and 0.52 mg of thiamin.

[3]Santoquin (Monsanto, St. Louis, MO) supplied 130 mg/kg ethoxyquin to the diets.

[4]Other includes Chromax (a source of Cr), choline chloride (60%), and copper sulfate supplied at 0.05, 0.10, 0.02 % of the lactation diet (as-fed basis) respectively.

4.3.3 Data and sample collection

4.3.3.1 Feed collection

Representative samples of corn, soybean meal, and mixed feed were collected at the feed mill for both phases of the experimental diets. Feed samples were stored at -20°C until analyzed.

4.3.3.2 Growth performance and blood collection

Body weight and blood samples were collected on d 0 (pre-injection), 1, 2, 3, 4, 6, 8, 11, 14, 17, and 22 (weaning) during the lactation period. After weaning, body and feeder weights were collected on d 23, 24, 25, 29, 38, 44, and 52. Blood samples were also collected at the same time points with the exception of d 44. The amount of feed added and discarded were recorded daily for each feeder.

For blood collection, only 50 of the 70 initial piglets (10 piglets per treatment) were used throughout the experiment. Blood samples were collected from each of the 50 pigs by jugular venipuncture. Blood was collected in 3 mL vacutainer tubes coated with K_2EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ) for complete blood count (CBC) analysis. Blood samples were immediately placed on ice and transported to the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL).

4.3.3.3 Tissue collection

A subset of pigs (3 pigs/treatment) was selected and sacrificed at weaning, d 38 (end of Phase I), and d 52 (end of Phase II) to evaluate tissue mineral concentrations. For this experiment, there was a total of 45 pigs that were euthanized by injection of sodium pentobarbital (SOCUMB, Henry Schein Animal Health, Dublin, OH). Following

euthanasia, pigs were dissected for selected tissues (liver, spleen, both kidneys, and heart) that were weighed, collected, and stored at -20°C until further analysis.

4.3.4 Sample processing and laboratory analysis

4.3.4.1 Experimental diet measures

For micro-minerals (Zn, Fe, and Cu) and calcium analysis, feed samples were first digested using a microwave digester (MARS 6, CEM Cooperation, Matthews, NC), then analyzed by flame atomic absorption spectrometry (Thermoelemental, SOLAAR Mf; Thermo Electron Corp., Verona, WI). Phosphorus content was analyzed using a gravimetric determination method (modification of method 968.08; AOAC, 1990).

4.3.4.2 Blood and tissue measures

Blood was analyzed at the UKVDL for a complete blood count (CBC) using a hematological analyzer (Forcyte Veterinary Hematology Analyzer, Oxford Science, Oxford, CT). Before analysis, all blood samples were thoroughly mixed and brought to room temperature. The CBC analysis consisted of hemoglobin concentration (Hb), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Tissue samples were placed through a kitchen-grade meat grinder (The butcher shop premium, KRUPS USA, Parsippany, NJ) to provide a homogenous tissue sample. After samples were ground and mixed, 1-2 g of tissue was digested with nitric acid in a pressurized microwave digester (MARS 6 CEM, Matthews, NC) according to the recommendations by the manufacturer, and appropriately diluted. Diluted samples were analyzed for trace mineral composition (Zn, Fe, and Cu) by flame atomic absorption

spectrophotometry (Thermoelemental, SOLAAR M5; Thermo Electron Corp., Verona, WI). Dry matter (DM) was determined for all tissue samples by placing around 2-3 g of ground sample into a gravity convection drying oven at 107°C for approximately 24 hours and weighing the sample again to observe the moisture content lost.

4.3.5 Statistical analysis

Prior to analyses, all data were evaluated to identify any potential statistical outliers detected by Grubb's test outlier calculator (Graphpad Software, San Diego, CA). All data for individual days were subjected to ANOVA by using the GLM procedure in SAS (Statistical Analysis System, Cary, NC). The individual pig was the experimental unit for individual BW and ADG, CBC measures, and tissue mineral concentrations. Results are reported as least squares means. Orthogonal polynomial contrasts were performed to evaluate the linear and quadratic effects of increasing iron injection dosage. Least squares means were calculated using the LSMEANS option of SAS. The level of significance was determined by a P-value of < 0.05. The data of growth performance, blood, and tissue measures for the pigs were analyzed by the model:

$Y_{ijk} = \mu + iron_i + litter_j + sex_k(litter_j) + e_{ijk}$, where,

Y = the response variables (ADG, blood CBC, organ weights, and tissue mineral concentrations)

μ = a constant common to all observations

$iron_i$ = the iron injection dosage level

$litter_j$ = the litter number

$sex_k(litter_j)$ = litter nested within sex

e_{ijk} = the error term of the model

For nursery pen performance, the pen served as the experimental unit by the model:

$Y_i = \mu + iron_i + e_i$, where

Y = the response variables (nursery ADG, ADFI, and F:G)

μ = a constant common to all observations

$iron_i$ = the iron injection dosage level

e_i = the error term of the model

Complete blood count (CBC) data were also analyzed as repeated measures with the PROC MIXED function of SAS to examine time and time by iron treatment interactions using the individual pig as the experimental unit by the model:

$Y_{ijkl} = \mu + iron_i + litter_j + sex_k(litter_j) + day_l + (iron \times day)_{il} + e_{ijkl}$, where

Y = the response variables (blood CBC measures)

μ = a constant common to all observations

$iron_i$ = the iron injection dosage level

$litter_j$ = the litter number

$sex_k(litter_j)$ = litter nested within sex

day_l = the day of sampling

$(iron \times day)_{il}$ = the iron injection dosage level × day of sampling interaction

e_{ijkl} = the error term of the model

4.4 Results

4.4.1 Growth performance

Pigs that did not receive an iron injection at birth had the lowest numerical BW by d 6 of the experiment, they continued to have the lowest BW during the rest of the sampling times (Table 4.2). This can be further explained by the low daily gains for the control pigs seen in Table 4.3. Weeks 1 and 3 following the iron injection at birth (Table 4.4) had a linear increase (P = 0.03 and P = 0.02) in ADG which led to a linear trend (P = 0.06) in heavier BW from d 8 to 17 (Table 4.2). By d 22 (weaning) there was a more noticeable linear response (P = 0.02) to iron injection dosage for BW which continued to the end of the experiment (d 52). Following weaning at Week 3 (Table 4.3), there was a clear linear increase (P < 0.01) with quadratic trends (P < 0.0001 and P = 0.07, respectively) in ADG for Weeks 4 and 5. When combined these results led to a noticeable linear and quadratic improvement (P < 0.001) in ADG during Phase I. Overall (Weeks 1 to 7), ADG was improved (P ≤ 0.02) in a linear and quadratic fashion in response to increasing iron injection dosage.

Nursery performance data from nursery pen means is provided in Table 4.5. Similar to the results demonstrated in Table 4.4, ADG was increased linearly (P ≤ 0.01) for Weeks 4 and 5. The improved ADG for Weeks 4 and 5 are accompanied by an increased (P ≤ 0.02, linear) ADFI at the same sampling times. During Phase I ADFI was increased (P = 0.01 and P = 0.02, respectively) both linearly and quadratically. In addition, ADFI for the nursery period was quadratically higher (P = 0.03); with a linear tendency (P = 0.07) as iron injection dosage increased.

Table 4.2. Effects of iron injection dosage on individual BW (kg)[1]

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of Pigs	14	14	14	13	14			
d 0	1.51	1.48	1.50	1.50	1.52	0.05	0.81	0.75
d 1	1.65	1.61	1.61	1.64	1.65	0.06	0.72	0.61
d 2	1.81	1.78	1.75	1.79	1.86	0.06	0.40	0.29
d 3	1.98	1.97	1.95	2.00	2.07	0.07	0.25	0.41
d 4	2.14	2.16	2.12	2.17	2.28	0.07	0.16	0.38
d 6	2.54	2.58	2.56	2.61	2.74	0.08	0.10	0.53
d 8	2.96	3.06	3.02	3.05	3.23	0.09	0.06	0.59
d 11	3.54	3.80	3.72	3.73	3.95	0.12	0.06	0.98
d 14	4.17	4.59	4.41	4.43	4.71	0.15	0.06	0.93
d 17	4.78	5.38	5.13	5.22	5.44	0.19	0.06	0.56
d 22 (weaning)	5.59	6.70	6.32	6.54	6.69	0.24	0.02	0.14
d 23[2]	5.26	6.62	6.57	6.56	6.49	0.23	0.01	<0.01
d 24	5.45	7.14	7.07	7.02	6.88	0.25	0.01	<0.001
d 25	5.62	7.51	7.45	7.47	7.24	0.26	<0.01	<.0001
d 29	6.99	9.14	9.18	9.29	8.93	0.30	<0.001	<.0001
d 38	11.08	14.33	14.36	14.36	14.46	0.46	<0.001	<0.001
d 44[3]	15.05	17.69	18.19	18.39	18.32	0.64	<0.01	0.01
d 52	20.45	23.09	24.34	23.66	23.74	0.75	0.02	0.01

[1]Iron injection treatments were administered on d 0, 1 pig from the 200 mg Fe injection treatment died on d 4 of the experiment.
[2]Means are represented by 11 pigs per treatment for all subsequent times until d 44.
[3] Means are represented by 8 pigs per treatment for all subsequent times.

Table 4.3. Effects of iron injection dosage on individual daily weight gain (g) during nursing and subsequent weaning period[1]

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of Pigs	14	14	14	13	14			
d 0-1	138.2	133.3	109.0	143.8	139.8	16.40	0.62	0.44
d 1-2	159.3	168.3	145.5	150.8	207.8	14.60	0.04	0.02
d 2-3	170.7	194.4	194.3	201.1	211.7	17.40	0.12	0.65
d 3-4	163.0	186.9	170.7	176.3	203.8	13.30	0.07	0.53
d 4-6	200.4	212.4	222.2	218.2	229.4	11.60	0.10	0.62
d 6-8	207.6	236.8	226.9	222.9	244.5	11.10	0.10	0.94
d 8-11	193.8	247.0	234.6	224.0	240.2	12.40	0.13	0.20
d 11-14	210.3	262.1	231.0	235.8	253.1	13.90	0.20	0.69
d 14-17	201.9	264.5	238.3	263.1	244.0	16.30	0.17	0.05
d 17-22	167.2	272.6	242.6	270.8	259.9	17.60	<0.01	0.01
d 22-23[2]	-168.1	-235.7	-171.0	-136.9	-249.6	45.00	0.56	0.28
d 23-24	192.7	516.7	493.7	454.7	390.3	60.75	0.23	<0.01
d 24-25	162.0	369.0	381.3	450.0	352.0	35.82	<0.01	<.0001
d 25-29	342.9	408.7	432.7	455.0	423.4	18.11	<0.01	<0.001

[1]Iron injection treatments were administered on d 0, 1 pig from the 200 mg Fe injection treatment died on d 4 of the experiment, pigs were weaned on d 22.
[2]Means are represented by 11 pigs per treatment for all subsequent times.

Table 4.4. Effects of iron injection dosage on individual pig average daily gain (ADG, g) [1]

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of Pigs	14	14	14	13	14			
Week 1	180.9	197.7	189.7	194.3	213.9	9.20	0.03	0.64
Week 2	202.0	254.6	232.8	229.9	246.7	11.82	0.12	0.36
Week 3	180.5	269.7	241.0	267.8	254.0	16.26	0.02	0.01
Week 4[2]	222.6	326.4	347.8	369.7	312.3	17.48	<0.01	<.0001
Week 5[2]	455.1	576.5	575.8	563.7	614.4	23.48	<0.001	0.07
Phase I (Wk 4-5)[2]	353.4	467.1	476.0	478.8	482.2	18.85	<0.001	<0.001
Week 6[3]	611.4	636.0	659.7	681.6	691.0	38.15	0.11	0.59
Week 7[3]	771.6	771.5	878.8	753.7	775.6	39.01	0.69	0.29
Phase II (Wk 6-7)[3]	697.7	709.0	777.7	720.4	736.5	33.54	0.55	0.35
Nursery period (Wk 4-7)[3]	516.3	569.9	609.4	590.7	585.3	21.28	0.06	0.02
Overall experiment[3,4]	372.2	424.2	448.6	434.4	436.5	14.34	0.02	0.01

[1]Iron injection treatments were administered on d 0, 1 pig from the 200 mg Fe injection treatment died on d 4 of the experiment, pigs were weaned at d 22.
[2]Means are represented by 11 pigs per treatment.
[3]Means are represented by 8 pigs per treatment.
[4]Overall experiment is representative of Weeks 1 through Week 7.

Table 4.5. Effects of iron injection dosage on nursery pen growth performance[1]

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of pens	3	3	3	3	3			
ADG, g								
d 22-29	213.9	313.0	333.4	359.1	302.9	18.47	0.01	<0.001
d 29-38	454.0	574.5	568.3	550.1	613.9	21.9	<0.01	0.18
Phase I (22-38)	349.0	460.1	465.5	466.5	477.8	17.24	<0.01	0.01
d 38-44	637.1	662.5	684.2	680.5	693.6	40.58	0.37	0.68
d 44-52	778.7	774.6	861.2	750.7	787.4	47.18	0.82	0.68
Phase II (38-52)	713.4	722.9	779.5	718.3	744.1	38.04	0.76	0.63
Nursery Period (22-52)	523.4	572.7	604.8	586.4	591.6	26.85	0.17	0.18
ADFI, g								
d 22-29	248.9	374.6	397.1	408.7	370.3	25.36	0.02	<0.01
d 29-38	591.0	749.1	750.5	731.6	787.6	33.28	0.01	0.11
Phase I (22-38)	420.0	561.8	573.8	570.2	578.9	26.96	0.01	0.02
d 38-44	907.8	985.7	1031.6	1024.7	1010.2	43.25	0.16	0.13
d 44-52	1086.5	1197.2	1277.8	1150.8	1170.1	63.02	0.79	0.19
Phase II (38-52)	997.2	1091.4	1154.7	1087.8	1090.1	47.29	0.41	0.12
Nursery Period (22-52)	708.6	826.6	864.3	829.0	834.5	31.66	0.07	0.03
F:G								
d 22-29	1.17	1.20	1.19	1.14	1.22	0.05	0.72	0.6
d 29-38	1.30	1.30	1.32	1.33	1.28	0.03	0.81	0.31
Phase I (22-38)	1.20	1.22	1.23	1.22	1.21	0.03	0.89	0.45
d 38-44	1.42	1.49	1.53	1.51	1.46	0.07	0.83	0.25
d 44-52	1.40	1.55	1.48	1.54	1.49	0.06	0.45	0.25
Phase II (38-52)	1.40	1.51	1.49	1.52	1.47	0.04	0.37	0.10
Nursery Period (22-52)	1.35	1.44	1.43	1.41	1.42	0.03	0.43	0.15

[1]Iron injection treatments were administered on d 0, 1 pig from the 200 mg Fe injection treatment died on d 4 of the experiment, pigs were weaned at d 22.

4.4.2 Hematological measures

Data are presented in a tabular manner (Table 4.6 – 4.12) as well as graphically (Figure 4.1 – 4.7). Absolute hemoglobin concentration (Table 4.6 and Figure 4.1) was lowest at all sampling times with the exception of d 52 for the pigs that did not receive any iron injection at birth. As early as d 1, hemoglobin concentration was improved ($P = 0.01$) with increasing iron injection dosage. However, by d 3 the improvement in hemoglobin was more pronounced as there was a linear and quadratic increase ($P < 0.0001$ and $P \leq 0.01$, respectively) that was observed through d 29. Both the 50 and 100 mg iron injection treatments had absolute hemoglobin concentrations that peaked at d 6 whereas the Hb concentration for the 200 and 300 mg iron treatments peaked at d 17. At d 38 of the experiment, there was still a quadratic increase ($P < 0.001$) in Hb concentration for iron treatments up to 50 mg iron but no improvement thereafter. By the end of the experiment (d 52) there were no differences observed with Hb concentration.

Similar to Hb concentration, HCT improved linearly ($P < 0.001$) and quadratically ($P < 0.01$) as iron dosage increased starting at d 3 continuing to d 29 (Table 4.7 and Figure 4.2). The improved HCT associated with iron injection treatment was unobserved at d 52 as all the treatments had similar HCT. The RBC measurement (Table 4.8 and Figure 4.3) did not demonstrate any clear relationship with iron treatment until d 3 when RBC was increased in a linear manner ($P = 0.05$). On d 4 there was both a linear and quadratic increase ($P = 0.03$ and $P = 0.05$, respectively) for RBC on increasing iron dosage. The linear and quadratic improvement of RBC was observed repeatedly through d 29, thereafter the differences between treatments become less noticeable and even similar by d 52.

Unlike the previous CBC measurements, WBC (Table 4.9 and Figure 4.4) showed a linear increase ($P < 0.05$) starting before the treatments were administered and lasting until d 29. At d 38 and 52 there were no differences in WBC for the five treatments. Mean corpuscular volume (MCV) (Table 4.10 and Figure 4.5) was greater ($P < 0.01$, linear) at d 3 and continued to be greater at all sampling times through d 29. Mean corpuscular hemoglobin (MCH) (Table 4.11 and Figure 4.6) was similar to MCV in showing a linear increase starting at d 3 ($P = 0.02$) and continuing to d 29. Differently, absolute MCHC (Table 4.12 and Figure 4.7) was numerically greater for pigs receiving no iron injection at d 6 through 14. At d 22 MCHC was greater ($P <0.0001$) as treatments increased, this observation continued on d 29. At d 38 MCHC showed a quadratic response ($P = 0.02$) to birth iron dosage.

Table 4.6. Effects of iron injection dosage on hemoglobin concentration (Hb, g/dL)[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
d 0	7.8	8.4	8.1	8.5	8.4	0.40	0.33	0.53
d 1	7.0	7.7	7.4	8.3	8.2	0.38	0.01	0.50
d 2	6.4	7.5	7.3	7.8	7.6	0.35	0.03	0.11
d 3	6.0	7.5	7.7	7.9	8.0	0.31	<0.001	0.01
d 4	5.6	7.7	8.0	8.1	8.2	0.27	<.0001	<.0001
d 6	5.0	8.2	9.2	9.6	9.6	0.23	<.0001	<.0001
d 8	4.5	7.8	9.1	9.8	9.4	0.33	<.0001	<.0001
d 11	4.2	7.6	9.2	10.8	11.0	0.19	<.0001	<.0001
d 14	4.0	7.3	8.8	11.2	11.8	0.21	<.0001	<.0001
d 17	3.8	7.2	8.8	11.3	12.2	0.29	<.0001	<.0001
d 22 (weaning)	3.8	7.2	8.5	10.9	12.1	0.42	<.0001	<.0001
d 23	3.7	7.3	8.5	11.1	12.0	0.38	<.0001	<.0001
d 24	3.7	7.4	8.8	11.2	12.1	0.42	<.0001	<.0001
d 25	3.8	7.3	8.4	10.5	11.3	0.36	<.0001	<.0001
d 29	6.3	9.9	10.4	11.2	11.0	0.38	<.0001	<.0001
d 38	9.5	11.1	11.1	11.0	10.7	0.30	0.07	<0.001
d 52[2]	11.2	11.7	11.3	11.5	11.2	0.28	0.74	0.39

[1]Iron treatments were administered after d 0 blood sampling.
[2]Treatment means reduced to 8 pigs per treatment.

Table 4.7. Effects of iron injection dosage on hematocrit percentage (HCT, %)[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
d 0	23.3	24.7	23.7	25.3	24.7	1.30	0.39	0.66
d 1	20.9	22.5	21.9	24.3	23.3	1.26	0.10	0.42
d 2	19.3	22.4	22.1	23.2	22.8	1.16	0.05	0.13
d 3	17.4	22.2	22.6	23.5	23.4	0.89	<0.001	<0.01
d 4	17.1	23.8	24.9	25.7	25.4	0.82	<.0001	<.0001
d 6	15.3	26.2	30.0	30.7	30.9	0.71	<.0001	<.0001
d 8	13.9	24.6	28.8	30.4	29.8	1.08	<.0001	<.0001
d 11	12.0	23.8	28.3	33.8	34.0	0.68	<.0001	<.0001
d 14	12.8	23.3	27.9	34.9	37.1	0.76	<.0001	<.0001
d 17	12.6	23.4	28.3	34.7	37.8	1.04	<.0001	<.0001
d 22 (weaning)	13.1	23.5	27.0	33.9	37.4	1.40	<.0001	<0.001
d 23	13.0	24.2	27.4	35.3	36.7	1.20	<.0001	<.0001
d 24	13.2	25.0	28.5	34.7	37.0	1.40	<.0001	<.0001
d 25	14.0	25.0	27.8	33.8	35.4	1.20	<.0001	<.0001
d 29	22.7	33.6	34.0	35.4	34.6	1.30	<.0001	<.0001
d 38	26.4	30.4	30.2	30.1	29.6	0.80	0.05	<0.01
d 52[2]	30.2	31.0	30.0	30.7	30.2	0.90	0.95	0.75

[1]Iron treatments were administered after d 0 blood sampling.
[2]Treatment means reduced to 8 pigs per treatment.

Table 4.8. Effects of iron injection dosage on red blood cell count (RBC, 10^6/µL)[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
d 0	3.50	3.84	3.73	4.03	3.88	0.20	0.14	0.29
d 1	3.22	3.63	3.47	3.94	3.67	0.21	0.08	0.17
d 2	3.05	3.46	3.34	3.56	3.43	0.19	0.20	0.24
d 3	2.89	3.33	3.26	3.48	3.37	0.16	0.05	0.12
d 4	2.91	3.34	3.37	3.54	3.41	0.15	0.03	0.05
d 6	2.71	3.71	3.89	4.00	3.98	0.17	<.0001	<0.001
d 8	2.68	3.90	4.05	4.13	4.21	0.19	<.0001	<0.01
d 11	2.45	4.14	4.39	4.70	4.79	0.18	<.0001	<.0001
d 14	2.90	4.45	4.78	5.08	5.38	0.19	<.0001	<.0001
d 17	3.07	4.84	5.29	5.44	5.77	0.24	<.0001	<0.01
d 22 (weaning)	3.44	5.49	5.69	5.81	5.98	0.29	<.0001	<0.001
d 23	3.37	5.68	5.83	6.12	5.96	0.24	<.0001	<.0001
d 24	3.44	5.87	6.08	6.01	6.05	0.26	<.0001	<.0001
d 25	3.40	5.63	5.82	5.86	5.78	0.24	<.0001	<.0001
d 29	4.10	6.21	6.22	5.94	5.55	0.23	0.01	<.0001
d 38	4.14	5.08	5.11	4.98	4.71	0.16	0.17	<.0001
d 52[2]	4.89	5.19	5.03	5.07	5.02	0.15	0.85	0.43

[1]Iron treatments were administered after d 0 blood sampling.
[2]Treatment means reduced to 8 pigs per treatment.

Table 4.9. Effects of iron injection dosage on white blood cell count (WBC, $10^3/\mu L$)[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
d 0	7.97	9.54	9.05	10.82	11.19	0.96	0.01	0.65
d 1	6.65	6.53	7.83	8.39	8.57	0.82	0.04	0.59
d 2	8.11	9.85	11.01	12.58	12.26	0.97	<0.01	0.10
d 3	9.49	11.03	12.26	13.69	12.58	0.98	0.01	0.05
d 4	9.76	11.51	12.99	14.71	12.98	0.95	0.01	0.01
d 6	8.43	9.70	11.02	12.58	10.76	0.77	0.01	0.01
d 8	7.62	8.08	8.56	9.59	9.23	0.49	0.01	0.20
d 11	6.41	7.43	7.76	8.67	9.17	0.48	<.0001	0.34
d 14	6.16	6.31	7.05	7.81	8.72	0.42	<.0001	0.93
d 17	6.50	6.27	6.92	7.36	8.65	0.66	0.01	0.48
d 22 (weaning)	6.93	5.68	6.60	7.42	10.05	1.13	0.01	0.15
d 23	6.95	6.34	8.29	9.29	12.55	1.46	<0.01	0.44
d 24	6.29	7.30	10.50	10.39	14.85	1.70	<0.001	0.91
d 25	7.04	7.73	8.87	9.50	13.93	1.44	<0.001	0.34
d 29	7.76	9.66	12.29	11.21	15.85	1.89	<0.01	0.98
d 38	12.07	13.29	11.59	12.51	14.15	1.28	0.31	0.43
d 52[2]	13.63	16.07	14.92	14.27	14.74	2.03	1.00	0.77

[1]Iron treatments were administered after d 0 blood sampling.
[2]Treatment means reduced to 8 pigs per treatment.

Table 4.10. Effects of iron injection dosage on mean corpuscular volume (MCV, fL)[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
d 0	66.4	64.2	63.5	62.8	63.6	0.98	0.05	0.07
d 1	64.8	62.3	62.9	61.9	63.6	1.09	0.56	0.09
d 2	63.1	65.1	66.4	65.8	66.4	1.21	0.10	0.23
d 3	60.4	67.7	70.0	68.8	69.6	1.54	<0.01	<0.01
d 4	58.9	72.6	74.8	73.7	74.8	1.83	<.0001	<.0001
d 6	57.7	72.2	78.2	77.8	78.2	2.29	<.0001	<.0001
d 8	53.4	64.5	72.2	74.8	71.0	2.24	<.0001	<.0001
d 11	53.0	58.2	64.9	72.5	71.3	2.13	<.0001	<0.01
d 14	46.7	53.0	58.7	69.1	69.2	1.83	<.0001	<0.01
d 17	43.2	48.6	53.6	64.3	65.7	1.78	<.0001	0.02
d 22 (weaning)	39.2	43.5	47.8	58.9	62.4	1.44	<.0001	0.07
d 23	39.4	43.1	47.1	58.2	61.4	1.46	<.0001	0.12
d 24	38.8	43.2	47.1	58.3	61.2	1.45	<.0001	0.06
d 25	41.3	44.8	48.0	58.3	61.4	1.44	<.0001	0.20
d 29	55.6	54.6	54.9	59.9	62.4	1.53	<.0001	0.25
d 38	63.9	60.2	59.3	60.6	62.9	1.07	0.90	<0.001
d 52[2]	61.7	59.8	59.8	60.7	60.3	0.92	0.67	0.36

[1]Iron treatments were administered after d 0 blood sampling.
[2]Treatment means reduced to 8 pigs per treatment.

Table 4.11. Effects of iron injection dosage on mean corpuscular hemoglobin (MCH, pg)[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
d 0	22.5	21.7	21.7	21.4	21.6	0.69	0.15	0.35
d 1	21.8	21.1	21.5	21.1	22.4	0.55	0.31	0.12
d 2	21.0	22.2	21.8	22.0	22.3	0.68	0.17	0.49
d 3	20.9	23.2	23.9	23.1	23.8	0.82	0.02	0.06
d 4	19.1	23.6	24.1	23.3	24.0	0.75	<0.001	<0.001
d 6	18.9	22.5	24.0	24.3	24.2	0.91	<0.001	<0.01
d 8	18.0	20.4	22.8	24.0	23.6	1.01	<0.01	<0.01
d 11	19.9	18.6	21.0	23.2	23.1	1.28	0.01	0.75
d 14	15.7	16.5	18.6	22.2	22.0	1.19	<.0001	0.22
d 17	13.9	15.0	16.8	21.0	21.2	0.97	<.0001	0.18
d 22 (weaning)	11.0	13.0	14.8	18.9	19.9	0.50	<.0001	0.01
d 23	11.0	12.7	14.5	18.3	20.1	0.45	<.0001	0.09
d 24	10.7	12.7	14.2	18.8	19.9	0.42	<.0001	0.01
d 25	11.0	13.0	14.3	18.0	19.6	0.40	<.0001	0.05
d 29	15.3	15.9	16.6	19.1	19.9	0.45	<.0001	0.60
d 38	22.7	21.7	21.6	22.1	22.6	0.41	0.91	0.03
d 52[2]	22.6	22.3	22.3	22.7	22.2	0.33	0.34	0.98

[1]Iron treatments were administered after d 0 blood sampling.
[2]Treatment means reduced to 8 pigs per treatment.

Table 4.12. Effects of iron injection dosage on mean corpuscular hemoglobin concentration (MCHC, g/dL)[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
d 0	34.0	33.9	34.5	34.2	34.0	1.09	0.88	0.70
d 1	33.8	34.1	34.3	34.3	35.6	0.73	0.11	0.74
d 2	33.3	34.1	32.8	33.4	33.6	0.57	0.83	0.69
d 3	34.6	34.2	34.1	33.7	34.2	0.58	0.40	0.24
d 4	32.5	32.4	32.2	31.7	32.2	0.41	0.31	0.27
d 6	32.9	31.2	30.7	31.3	30.9	0.45	0.04	0.04
d 8	33.3	31.6	31.6	32.0	31.8	0.70	0.22	0.37
d 11	36.4	32.0	32.4	32.1	32.4	1.15	0.05	0.03
d 14	32.4	31.2	31.7	32.1	31.9	1.11	0.99	0.72
d 17	31.4	30.9	31.3	32.7	32.2	0.85	<.0001	0.18
d 22 (weaning)	28.3	30.4	31.2	32.1	32.1	0.40	<.0001	<0.001
d 23	28.2	29.9	31.1	31.5	32.8	0.36	<.0001	0.04
d 24	28.0	29.7	30.7	32.3	32.7	0.31	<.0001	<0.01
d 25	27.2	29.4	30.3	31.0	32.0	0.29	<.0001	<0.001
d 29	27.9	29.4	30.7	31.7	32.0	0.29	<.0001	<.0001
d 38	35.9	36.4	36.7	36.4	36.1	0.33	0.92	0.02
d 52[2]	36.9	37.6	37.7	37.3	36.9	0.28	0.37	0.08

[1]Iron treatments were administered after d 0 blood sampling.
[2]Treatment means reduced to 8 pigs per treatment.

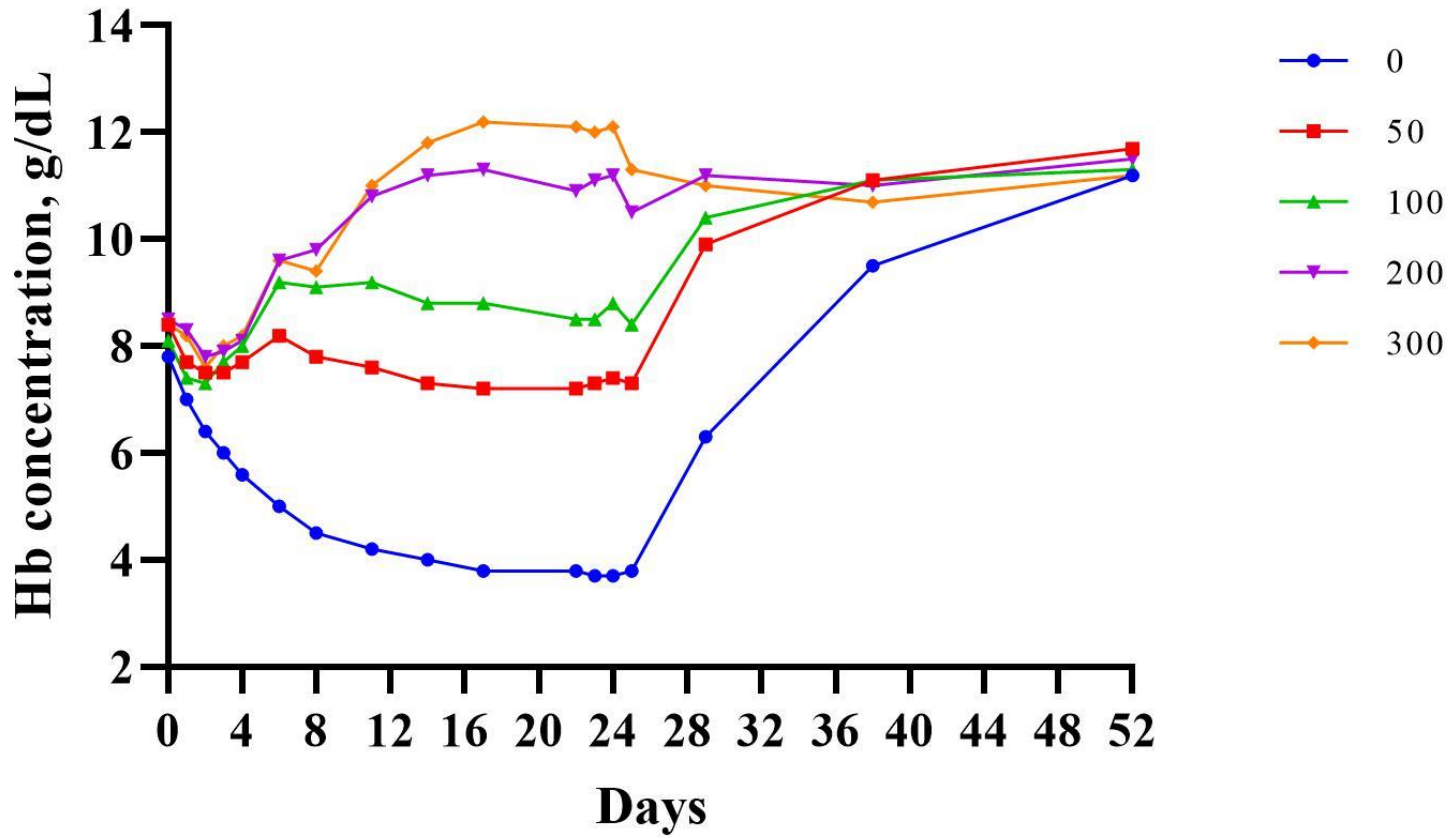

Figure 4.1. Effects of iron injection dosage on hemoglobin (Hb) concentration. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P < 0.0001; Day, P < 0.0001; Trt*Day, < 0.0001; Day contrast: Linear, P < 0.0001 and quadratic < 0.0001.

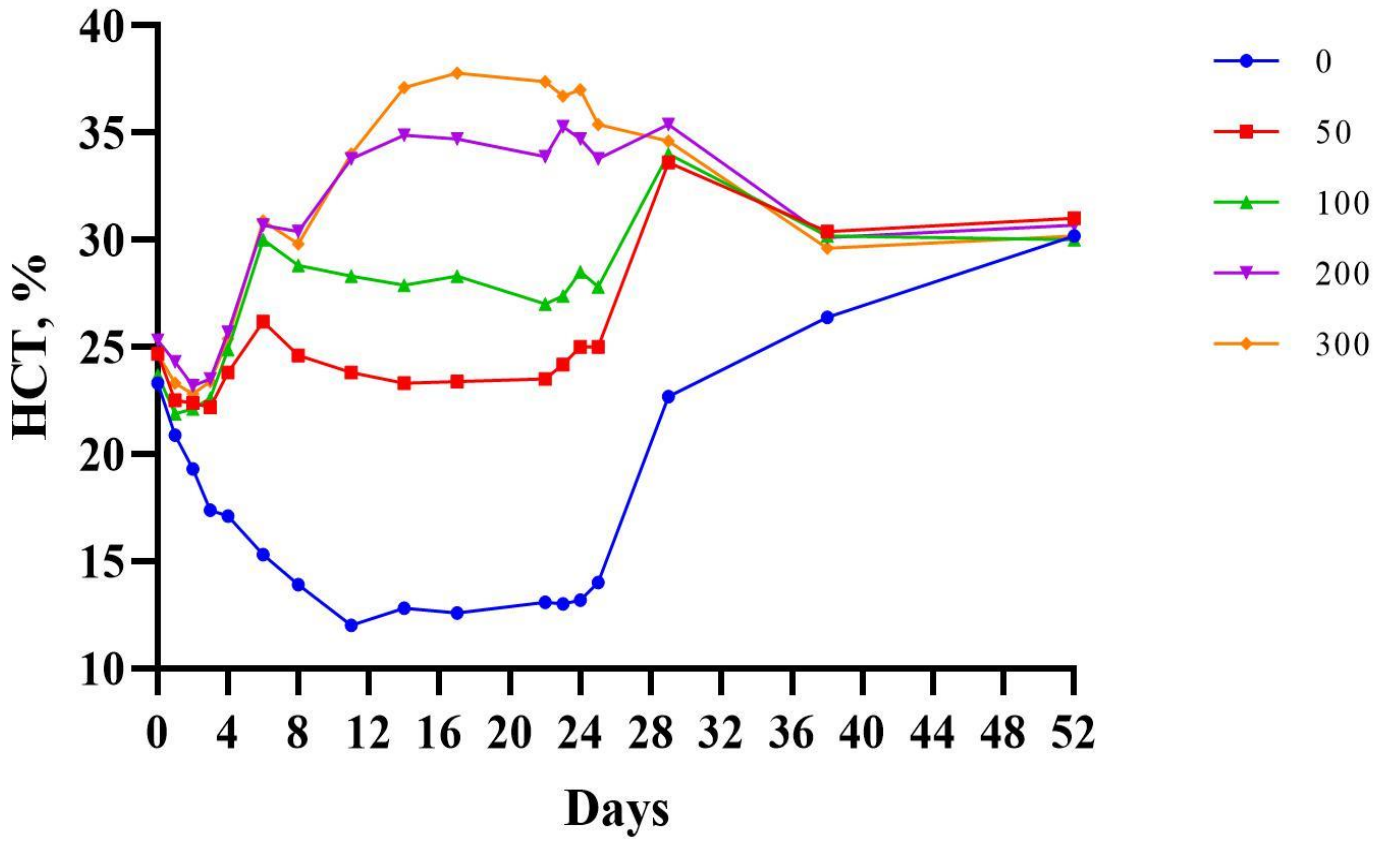

Figure 4.2. Effects of iron injection dosage on hematocrit content (HCT). Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P < 0.0001; Day, P < 0.0001; Trt*Day, < 0.0001; Day contrast: Linear, P < 0.0001 and quadratic < 0.0001.

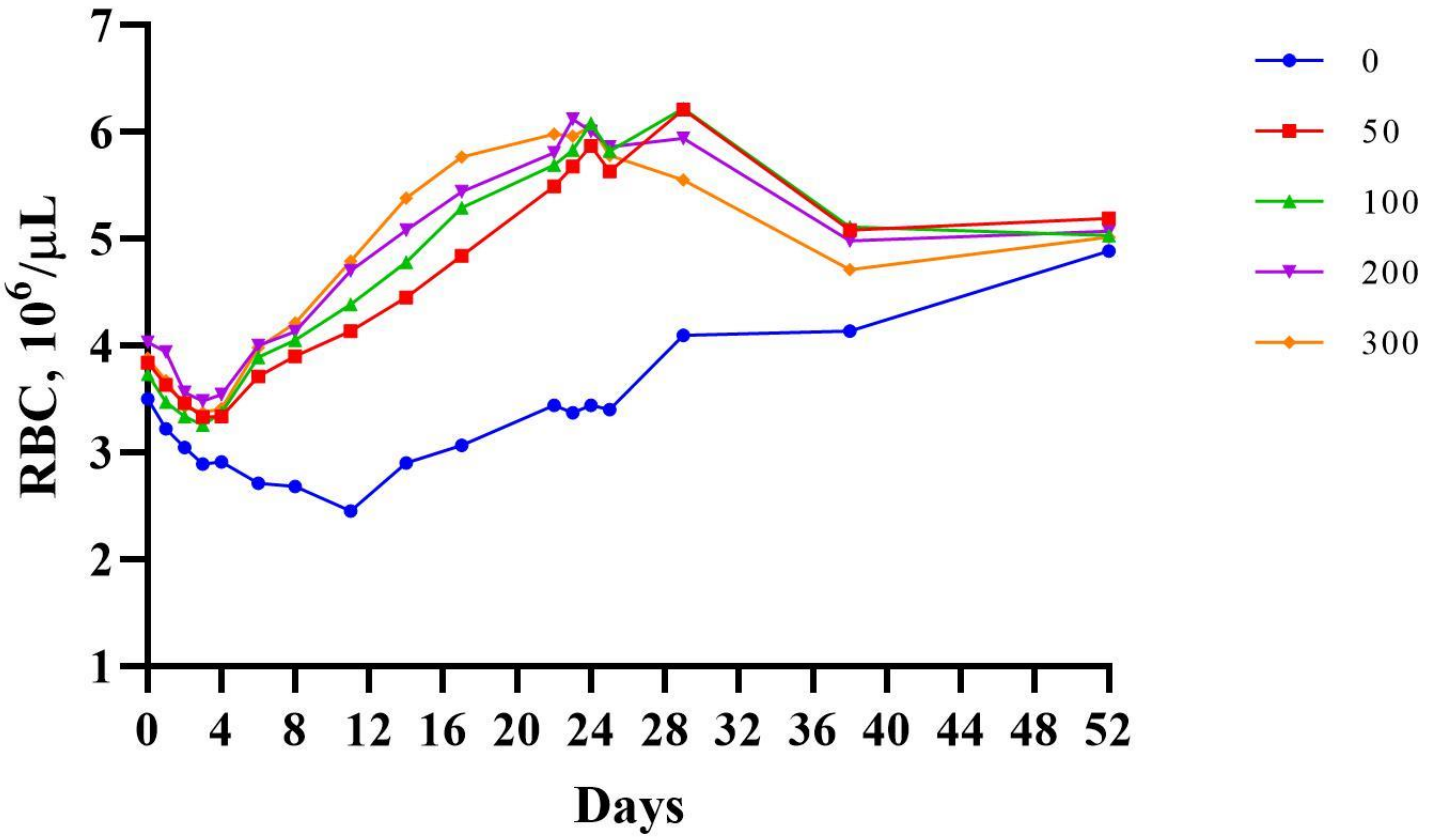

Figure 4.3. Effects of iron injection dosage on red blood cell count (RBC). Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P < 0.0001; Day, P < 0.0001; Trt*Day, < 0.0001; Day contrast: Linear, P < 0.0001 and quadratic < 0.0001.

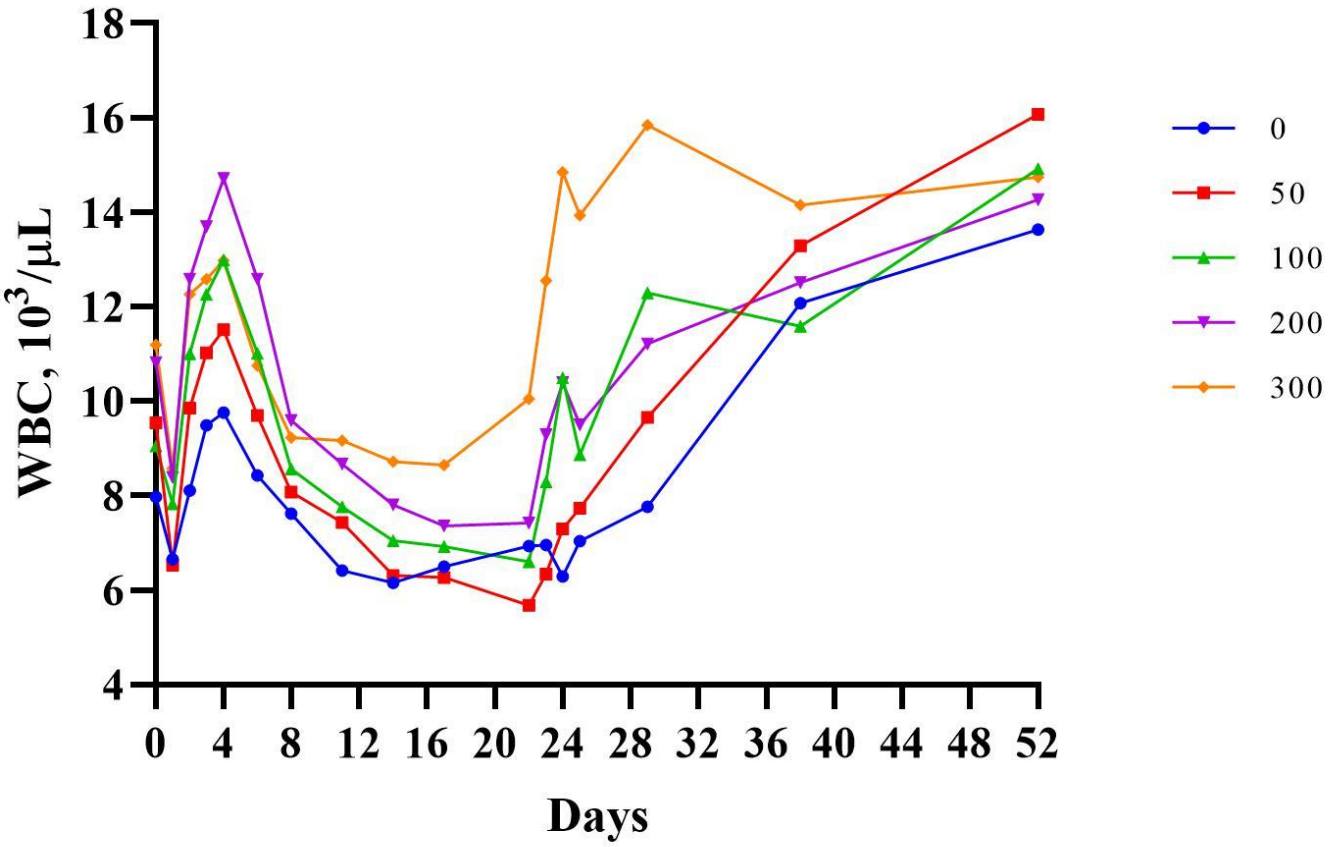

Figure 4.4. Effects of iron injection dosage on white blood cell count (WBC). Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P < 0.001; Day, P < 0.0001; Trt*Day, < 0.0001; Day contrast: Linear, P < 0.0001 and quadratic < 0.0001.

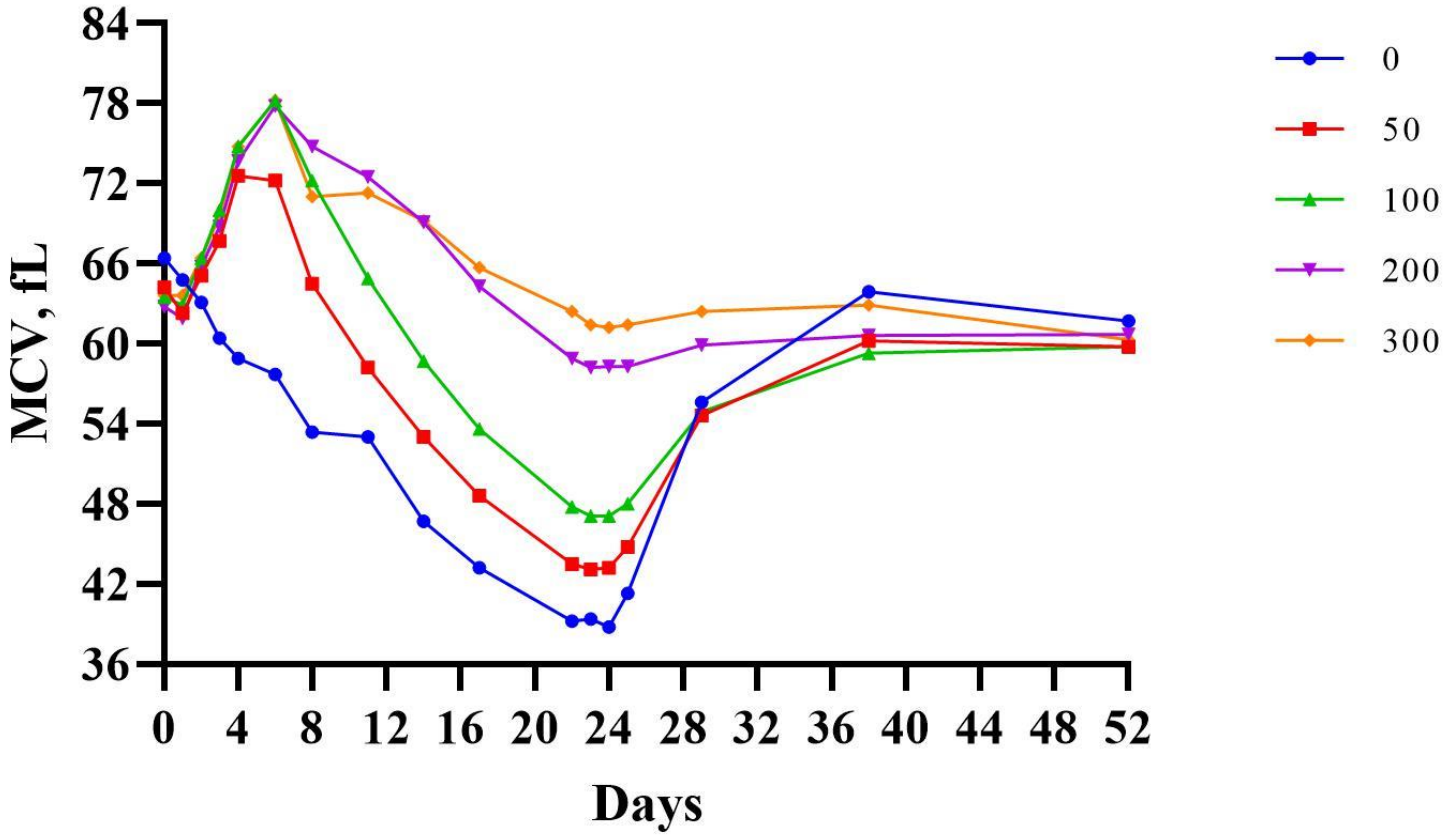

Figure 4.5. Effects of iron injection dosage on mean corpuscular volume (MCV). Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P < 0.0001; Day, P < 0.0001; Trt*Day, < 0.0001; Day contrast: Linear, P < 0.0001 and quadratic < 0.0001.

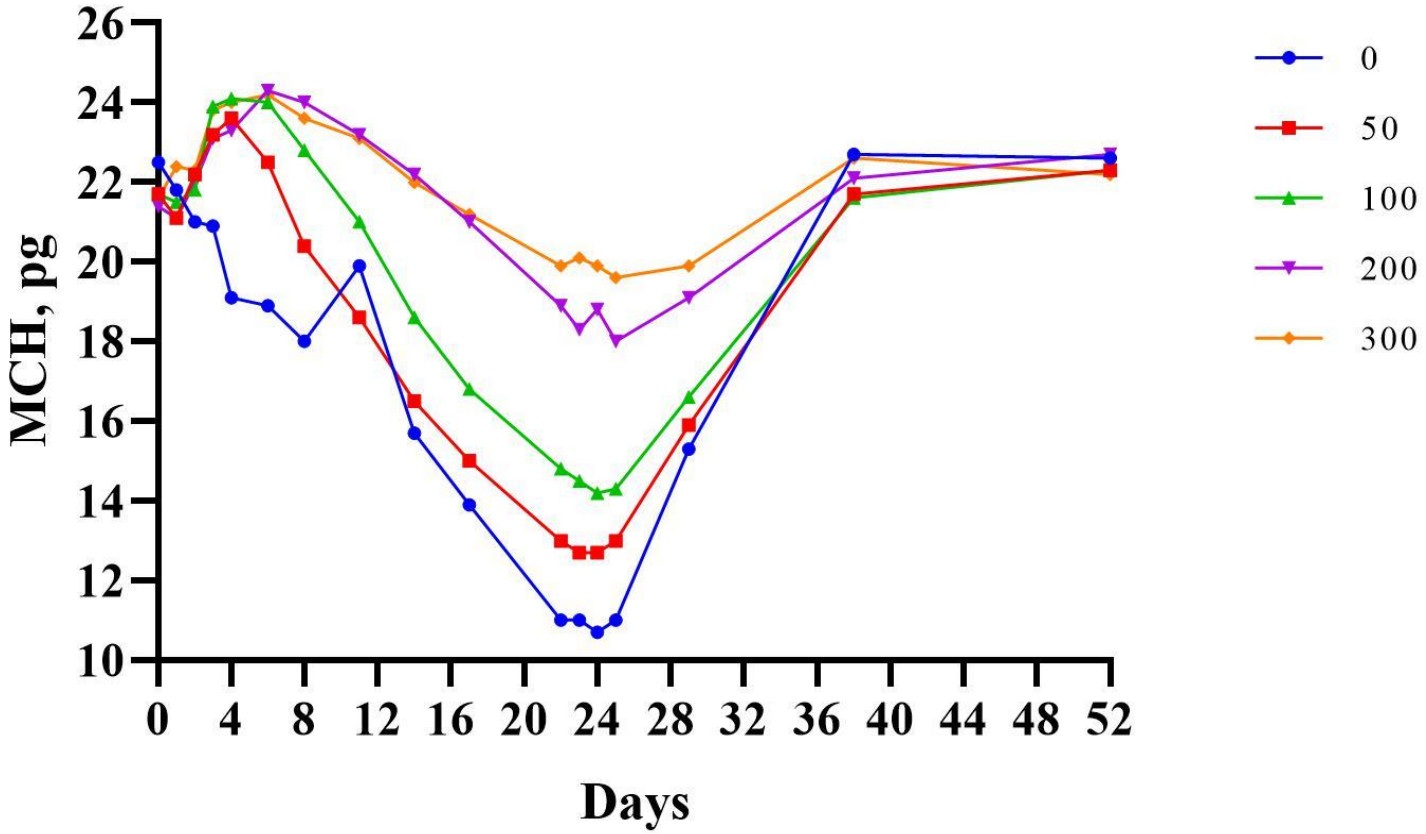

Figure 4.6. Effects of iron injection dosage on mean corpuscular hemoglobin (MCH). Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P < 0.0001; Day, P < 0.0001; Trt*Day, < 0.0001; Day contrast: Linear, P < 0.0001 and quadratic < 0.0001.

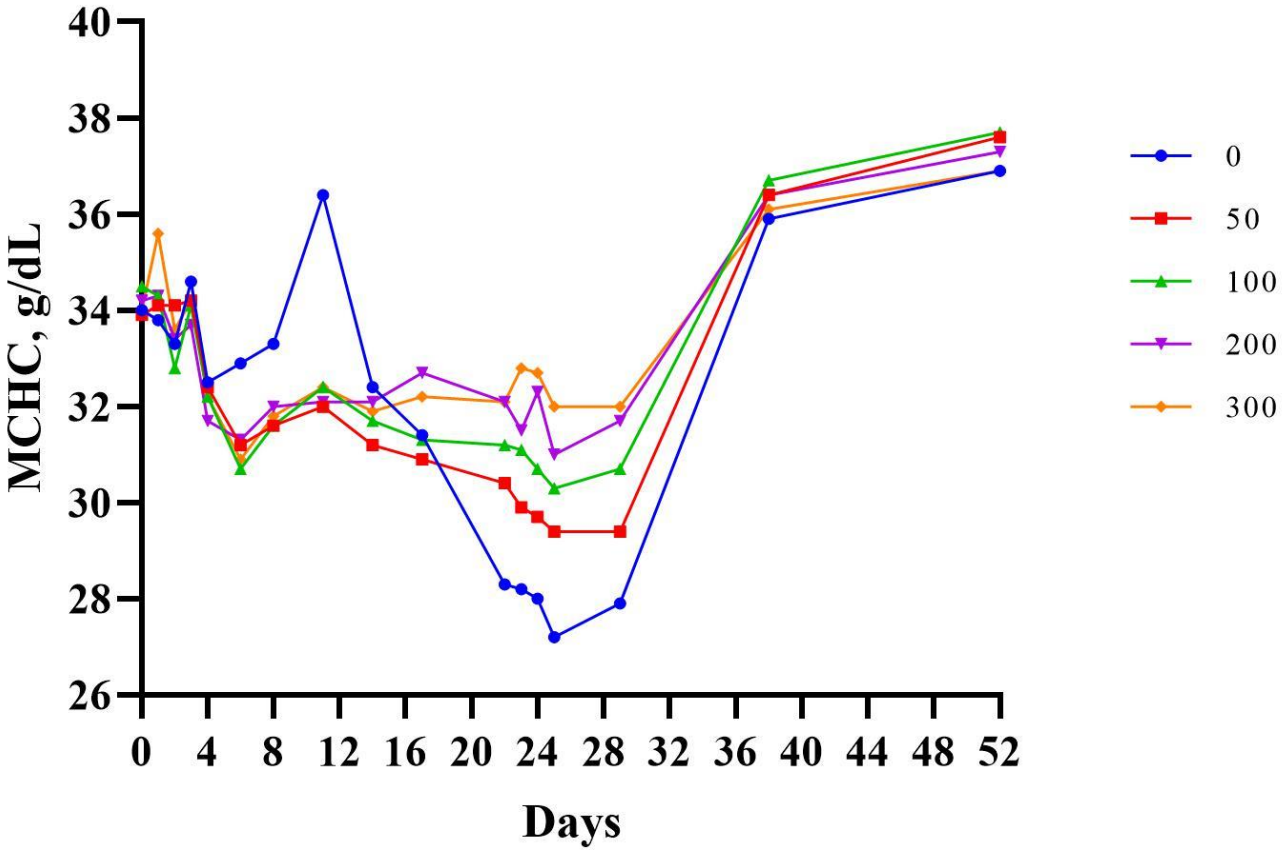

Figure 4.7. Effects of iron injection dosage on mean corpuscular hemoglobin concentration (MCHC). Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P < 0.011; Day, P < 0.0001; Trt*Day, < 0.0001; Day contrast: Linear, P < 0.0001 and quadratic < 0.0001.

4.4.3 Tissue measures

A total of 3 pigs per treatment per sampling period were used to determine the mineral concentration of liver, spleen, heart, and kidney. Tissue mineral concentrations are reported on a DM basis. Throughout the experiment, the average DM content for liver, spleen, heart, and kidney were 22.2, 19.5, 18.3, and 17.1 % respectively.

Liver iron concentration (Table 4.13) was higher in response to increasing iron injection dosage at weaning (d 22) and d 38 (end of Phase I) ($P < 0.01$ and $P = 0.02$; respectively). Also at weaning, the 300 mg iron treatment had liver iron concentrations about 17 times greater than the pigs not receiving iron. Liver zinc concentration increased ($P = 0.01$) with increasing iron treatments at d 52 (end of Phase II).

Similar to the liver, at weaning the spleen (Table 4.14) exhibited an increase in iron concentration ($P < 0.01$), but differently there was a decrease in spleen zinc content ($P = 0.03$) as iron injection dosage increased with a tendency ($P= 0.08$) to decrease quadratically with the 200 mg iron treatment having the largest reduction which thereafter was increased. At d 38 the relative weight of the spleen decreased ($P = 0.02$) as treatments increased. Interestingly at d 52 (end of Phase II), an increase ($P = 0.04$) in spleen iron content as iron dosage increased was observed again. Also at d 52, there was a numerical decrease in spleen zinc content through the 200 mg iron treatment then an increase was observed for the 300 mg iron treatment. Notably, over the tissue collection periods of the experiment (weaning, d 38, and d 52), a decrease in mean zinc and copper concentration was observed for both the liver and spleen in contrast to iron which increased with time.

Similar to liver and spleen there was an increase in iron content ($P = 0.01$) of the heart as iron injection increases (Table 4.15). Moreover, at weaning, there was a linear and quadratic decrease ($P < 0.05$) in the absolute and relative weight of the heart as iron treatments increased. The linear and quadratic effects of decreasing relative heart weight with increasing iron treatment continued to d 38 ($P = 0.01$, $P < 0.01$; respectively) but by d 52 there were no differences in heart size. The pigs receiving no iron had the heaviest absolute and relative heart weights at both weaning and d 38. Finally, kidney iron concentration at weaning was also elevated ($P < 0.0001$). Interestingly zinc and copper concentration were reduced ($P = 0.02$) quadratically as iron treatments increased to 200 mg iron but thereafter zinc and copper concentration began to increase for pigs supplied 300 mg iron (Table 4.16). These effects of kidney zinc and copper disappeared at d 38 where there were no differences between treatments. However, at d 52 kidney zinc concentration increased ($P = 0.02$) quadratically from 0 mg to 200 mg iron. Although not significant, at d 52 kidney copper concentration had a numerical increase similar to that of kidney zinc where the 200 mg iron treatment had the greatest zinc and copper concentrations.

Table 4.13. Effects of iron injection dosage on liver mineral content (mg/kg DM)

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of Pigs	3	3	3	3	3			
Weaning (d 22)								
BW, kg	6.39	6.61	5.03	6.20	6.04	0.53	0.71	0.37
Liver WT, g	193.03	223.70	159.17	213.15	214.97	18.46	0.44	0.48
Liver WT, % BW	3.03	3.40	3.18	3.43	3.58	0.21	0.13	0.91
Fe	95.8	143.0	204.5	402.9	1652.5	348.73	<0.01	0.15
Zn	287.8	247.6	296.9	211.5	276.0	23.75	0.47	0.26
Cu	413.6	415.6	482.8	448.1	413.7	56.34	0.99	0.43
d 38								
BW, kg	9.73	14.94	14.30	13.84	14.51	1.16	0.08	0.09
Liver WT, g	367.40	602.57	536.17	538.03	570.47	67.93	0.20	0.26
Liver WT, % BW	3.72	3.99	3.74	3.89	3.91	0.25	0.72	0.94
Fe	380.1	627.1	586.5	610.8	654.2	57.61	0.02	0.13
Zn	146.0	137.8	117.2	100.8	107.4	17.26	0.08	0.35
Cu	159.6	73.9	99.9	121.9	85.7	29.48	0.38	0.58
d 52								
BW, kg	21.19	22.27	24.49	22.53	22.61	2.38	0.80	0.52
Liver WT, g	803.00	806.83	870.63	865.17	828.57	90.84	0.78	0.60
Liver WT, % BW	3.80	3.63	3.56	3.84	3.66	0.11	0.99	0.69
Fe	742.4	796.5	819.6	786.0	913.4	90.74	0.27	0.81
Zn	114.5	129.5	132.6	182.1	180.2	17.78	0.01	0.58
Cu	35.5	25.2	35.1	33.3	30.1	3.81	0.80	0.90

Table 4.14. Effects of iron injection dosage on spleen mineral content (mg/kg DM)

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of Pigs	3	3	3	3	3			
Weaning (d 22)								
BW, kg	6.39	6.61	5.03	6.20	6.04	0.53	0.71	0.37
Spleen WT, g	15.63	18.43	16.83	25.15	17.67	3.37	0.39	0.25
Spleen WT, % BW	0.25	0.28	0.33	0.40	0.28	0.06	0.37	0.09
Fe	483.6	665.1	983.0	1189.3	1149.8	134.59	<0.01	0.10
Zn	83.5	81.7	73.4	62.7	72.8	4.53	0.03	0.08
Cu	7.3	8.2	6.0	6.3	6.2	0.96	0.17	0.58
d 38								
BW, kg	9.73	14.94	14.30	13.84	14.51	1.16	0.08	0.09
Spleen WT, g	31.17	41.13	34.03	33.63	34.67	4.19	0.91	0.70
Spleen WT, % BW	0.32	0.27	0.24	0.24	0.24	0.02	0.02	0.07
Fe	581.9	692.1	643.4	713.4	644.9	84.13	0.66	0.42
Zn	56.0	67.5	59.4	56.1	56.5	6.60	0.58	0.72
Cu	4.4	4.5	3.8	3.7	3.7	0.40	0.16	0.49
d 52								
BW, kg	21.19	22.27	24.49	22.53	22.61	2.38	0.80	0.52
Spleen WT, g	54.13	82.53	82.37	74.30	84.90	15.80	0.38	0.53
Spleen WT, % BW	0.28	0.37	0.33	0.33	0.39	0.08	0.51	0.98
Fe	774.7	1382.6	1125.7	1308.5	1571.7	194.75	0.04	0.68
Zn	63.6	44.6	61.0	48.1	66.0	7.05	0.60	0.13
Cu	3.7	4.6	4.5	3.8	5.4	0.41	0.07	0.41

Table 4.15. Effects of iron injection dosage on heart mineral content (mg/kg DM)

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of Pigs	3	3	3	3	3			
Weaning (d 22)								
BW, kg	6.39	6.61	5.03	6.20	6.04	0.53	0.71	0.37
Heart WT, g	57.50	46.07	32.97	42.90	38.67	3.46	0.01	0.02
Heart WT, % BW	0.90	0.70	0.66	0.70	0.65	0.03	<0.01	0.01
Fe	163.19	190.56	341.71	283.96	379.09	50.11	0.01	0.49
Zn	56.60	61.33	72.03	65.09	53.32	7.64	0.68	0.13
Cu	10.55	12.48	13.84	11.79	10.72	1.18	0.65	0.11
d 38								
BW, kg	9.73	14.94	14.30	13.84	14.51	1.16	0.08	0.09
Heart WT, g	72.47	86.23	77.67	73.43	81.33	7.25	0.86	0.97
Heart WT, % BW	0.75	0.58	0.54	0.53	0.56	0.03	0.01	<0.01
Fe	222.72	281.58	247.58	243.68	259.03	28.29	0.75	0.76
Zn	59.29	60.09	55.09	47.95	55.48	2.99	0.08	0.10
Cu	11.95	13.83	13.97	11.83	13.47	0.68	0.82	0.67
d 52								
BW, kg	21.19	22.27	24.49	22.53	22.61	2.38	0.80	0.52
Heart WT, g	111.40	108.47	121.20	118.00	115.23	12.89	0.74	0.67
Heart WT, % BW	0.55	0.49	0.49	0.53	0.51	0.05	0.88	0.64
Fe	294.68	357.50	345.50	380.64	320.40	33.82	0.65	0.12
Zn	53.81	47.37	46.79	52.34	49.40	3.44	0.88	0.51
Cu	16.16	14.65	14.08	15.87	14.87	1.46	0.87	0.69

Table 4.16. Effects of iron injection dosage on kidney mineral content (mg/kg DM)

	Iron injection, mg Fe						Contrast	
Variable	0	50	100	200	300	SEM	L	Q
No. of Pigs	3	3	3	3	3			
Weaning (d 22)								
BW, kg	6.39	6.61	5.03	6.20	6.04	0.53	0.71	0.37
Kidney WT, g	41.67	43.77	28.50	41.10	34.73	3.83	0.32	0.50
Kidney WT, % BW	0.65	0.66	0.56	0.66	0.58	0.04	0.39	0.99
Fe	96.3	213.5	301.1	433.8	544.8	35.46	<.0001	0.23
Zn	76.5	70.9	66.3	63.7	69.0	2.54	0.05	0.02
Cu	38.0	37.4	19.9	18.9	24.5	3.61	0.01	0.02
d 38								
BW, kg	9.73	14.94	14.30	13.84	14.51	1.16	0.08	0.09
Kidney WT, g	70.43	91.87	95.37	88.77	101.57	10.10	0.12	0.50
Kidney WT, % BW	0.72	0.61	0.68	0.64	0.70	0.04	0.95	0.21
Fe	274.2	421.9	422.5	439.7	388.3	45.99	0.21	0.04
Zn	73.1	72.5	72.6	66.4	68.5	3.02	0.14	0.68
Cu	30.1	26.5	28.5	22.3	25.1	4.17	0.32	0.58
d 52								
BW, kg	21.19	22.27	24.49	22.53	22.61	2.38	0.80	0.52
Kidney WT, g	133.57	139.37	169.23	144.10	139.63	20.26	0.96	0.36
Kidney WT, % BW	0.63	0.63	0.68	0.63	0.63	0.05	0.93	0.62
Fe	547.8	493.2	504.5	454.4	555.2	57.49	0.98	0.23
Zn	61.9	72.8	64.2	79.1	67.9	2.64	0.07	0.02
Cu	23.9	26.5	22.0	41.1	32.2	5.40	0.09	0.52

4.5 Discussion

4.5.1 Growth performance

Increasing iron injection dosages at birth resulted in increased growth performance during both the lactation and nursery periods. The improved growth in the present experiment was mostly noticed at Week 3, which was the week of weaning, and the first 2 weeks of the nursery period (Week 4 and 5). The days leading up to weaning (d 17 to 21) have been shown to be important in regard to hematological measures declining and to levels lower than initial values (Holter et al., 1991). It has also been observed that optimal iron status (Hb > 11g/dL) at weaning may lead to improved growth performance in the subsequent nursery period (Fredericks et al., 2018). The positive growth performance that may be associated with optimal iron status is attributed to improved oxygen transport, immune function, vitality, and metabolism (Von der Recke et al., 2014).

Overall, pigs not receiving an iron supplement demonstrated numerically and statistically the lowest growth performance. This poor growth performance from the 0 mg iron injection group is accompanied by low CBC and tissue mineral concentrations indicating that iron-deficient anemia was induced. There was a linear increase ($P = 0.03$) in ADG during Week 1 as the iron dosage increased, which was later observed in Week 3 with a quadratic increase ($P = 0.01$) as well. Williams et al. (2018) demonstrated only a quadratic ($P = 0.002$) improvement in ADG with increasing iron dosage from 0 to 50 mg iron and no further improvement thereafter for the lactation period after being administered various amounts of injectable iron (0, 50, 100, 150, and 200 mg iron).

4.5.2 Hematological measures

As expected, before the administration of iron treatments all CBC measurements with the exception of WBC were similar between treatments. Unexpectedly, WBC results were different before any of the treatments were administered. Although there was a difference, all means were within the reference ranges described by Perri et al. (2017).

In the current experiment, increasing the dosage of injectable iron at birth led to increasing CBC measurements (Hb, HCT, RBC, WBC, MCV, MCH, and MCHC) throughout the experiment. For the most part, the time course of Hb, HCT, RBC, MCV, and MCH were very similar. These measurements tended to noticeably differ around d 3 post-injection, continuing to differ until d 29. However, by d 38 the differences between treatments were less clear than the previous days, and by d 52 the differences that were once present disappeared. Williams et al. (2018) reported linear and quadratic increases ($P = 0.001$) of Hb concentrations and HCT on d 11 and 21 in response to increasing iron dosage (0, 50, 100, 150, and 200 mg iron). These findings agree with the present study; however, Williams et al. (2018) also reported a linear increase ($P = 0.001$) in Hb and HCT observed on d 35. In contrast with the previous work, on d 38 Hb and HCT increased from iron treatments 0 to 50, but thereafter remained similar. Notably, the nursery diets used by Williams et al. (2018) contained similar analyzed levels of iron in the diet compared to the present experiment (255 ppm vs 220 ppm iron). In the present experiment, absolute Hb concentration for the 200 and 300 mg iron treatments peaked at 17 days after administration of the injection where it then began to decline. This is in agreement with the theoretical model proposed by Van Gorp et al. (2012), suggesting that the iron supply from the initial iron injection will only last approximately 17 to 18 days.

Egeli et al. (1998) reported normal and anemic values for HCT, RBC, MCV, MCH, and MCHC at 21 d after receiving or not receiving an iron injection. In agreement with Egeli et al. (1998), only the 300 mg iron treatment in the present experiment was equal to or above their normal HCT (37% vs. 34%) and MCH (19.9 pg vs. 19.2 pg) values which were observed from pigs receiving 180 mg iron at processing, while all the treatments in the current study with the exception of 0 mg iron were above the RBC and MCHC values (> 5.35 M/μL, > 30.2 g/dL; respectively). In contrast, all of the treatments in the current experiment were below the MCV value (63.6) reported by Egegli et al. (1998). Notably, in the experiment reported by Egegli et al. (1998) they offered a creep feed as an additional supplement during the lactation period in contrast to no creep feed being offered in the current study which could possibly explain some differences between the two studies.

White blood cell count also was higher as the iron treatments increased at similar time points as the RBC measurements. WBC is often used with other indicators to evaluate immune function. However, because white blood cell count is a total measurement of all white blood cells which includes neutrophils, basophils, eosinophils, monocytes, and lymphocytes it can be deceptive by concluding anything simply on total WBC values. Therefore the reduction seen in WBC for treatments containing no or low doses of iron could indicate a possible increase in susceptibility to infection, but without the measurements of the individual components of white blood cells this assumption is uncertain.

Different from all the other CBC measurements, mean corpuscular hemoglobin concentration (MCHC) decreased in response to increasing iron dosage from d 6 to 11.

More so from d 17 to 29, the results were completely opposite in which MCHC increased with increasing iron. Mean corpuscular hemoglobin concentration is the concentration of hemoglobin within the RBC usually indicating the oxygen-carrying capacity of the blood. Data reported by Egegli et al. (1998) demonstrated that anemic pigs supplied with no iron at birth have higher MCHC values than normal pigs which received an iron injection. This would explain the decrease in MCHC observed with increasing iron in the current experiment from d 6 to 11. Due to the lower RBC number for pigs not receiving iron at birth the body may be compensating by loading hemoglobin within the red blood cells that are present. Later on from d 17 through 29 there was an increase in MCHC with increasing iron dosage, this improvement is simply explained by the other improvements in CBC measurements associated with increasing iron dosage which all can contribute to improved hematological measures. The elevated MCHC at d 6 to 11 for pigs not receiving iron at birth is in disagreement with current literature explaining that iron-deficient anemia is defined by hypochromic red blood cells (Dallman, 1986; Szudizik et al., 2018). Due to the high MCHC content, the red blood cells will appear a darker color because of the higher hemoglobin concentration within the red blood cells. This is particularly interesting because, from the current data, it seems that the pigs receiving 0 mg iron at birth were demonstrating a biological compensation for the lack of iron until it is physically incapable of doing so (after d 11).

4.5.3 Tissue measures

In the current experiment, there was an increase in the iron concentration of the liver, spleen, heart, and kidneys at weaning when the injectable iron dose at birth increased. The liver and spleen are major sites for ferritin and hemosiderin which are iron storage

compounds (Dallman, 1986). Thus it is explained why there was a linear response to iron treatments for liver and spleen concentration at weaning. Iron transport through the body is dependent on the transport protein transferrin. Transferrin delivers iron at a rate dependent on the pace of RBC production which is dependent on the overall iron status of the individual (Huebers and Finch, 1984). This concept may explain why in the present study there were greater concentrations of iron in tissues of those receiving greater iron dosages.

At weaning, the heart was larger for pigs receiving no supplemental iron. Due to the low amount of hemoglobin or oxygen in the blood of anemic pigs, it is proposed that the heart has to compensate and increase output to deliver more blood (oxygen) to tissues. These results are in agreement with an explanation by Dallman (1986) describing that severe anemia leads to cardiac hypertrophy which is what was observed at weaning in the current experiment.

Also at weaning, the zinc concentration of the spleen and kidney reduced as injectable iron dosage increased. Iron and zinc have been known to have competitive interaction for cellular transport especially when there are elevated iron levels (Solomons and Jacob, 1981). More interestingly, Camaschella and Pagani (2018) demonstrated that with higher iron concentrations in the body, zinc transporter protein 14 (ZIP14) will transport iron in hepatocytes and other cells which is in agreement with the current data. This could also explain the trend for a decrease ($P = 0.08$) in liver zinc concentrations observed at d 38, which later became an increase ($P = 0.01$) in liver zinc by the end of the experiment (d 52). Biologically at d 38, the liver and hepatocytes may still be processing the higher iron concentrations observed at weaning, but once the iron concentrations are under control

(observed at d 38) the liver and hepatocytes can then start to compensate for the lower zinc concentrations leading to the improvement in zinc concentrations by d 52.

4.6 Conclusion

The results from the current experiment demonstrated that without an iron supplement given at birth pigs become iron deficient which leads to iron-deficient anemia. Improved growth performance and CBC measurements were observed with greater injectable iron dosages administered at birth. These data are in agreement with literature suggesting that an initial supplement of iron given to newborn piglets is vital for the growth and ability to thrive in later periods of life. In regard to iron injection dosage in this experiment, the 50, 100, and 200 mg iron treatments were somewhat similar. However, the 300 mg iron treatment seemed to have a more consistent improvement in iron status throughout the various times of the experiment.

CHAPTER 5. Effects of an additional iron injection administered 4 days before weaning on growth performance, hematological status, and tissue mineral concentrations of nursery pigs

5.1 Abstract

The objective of the present experiment was to evaluate the effects of administering an additional iron injection 4 days before weaning on growth performance, hematological status, and tissue mineral concentration pre and postweaning. A total of 136 crossbred pigs (14 to 20 d; initial BW of 5.48 ± 1.08 kg) were selected in pairs that were within a litter, the same sex, and a BW difference of < 0.41 kg and then assigned to either a control or treatment group. All pigs received an initial intramuscular (IM) injection of iron dextran (150 mg iron) at d 1 after birth. Pigs that were assigned to the treatment group received an additional 150 mg iron injection 4 days before weaning (14 to 20 d), in contrast to the control group which received no additional iron injection at this time. Pigs were then weaned 4 days later (at 18 to 24 days) to nursery pens where they were fed a common two-phase nursery diet. The common nursery diets were formulated to meet or exceed the NRC (2012) nutrient requirement estimates for pigs 7 to 25 kg. Pigs were weighed at d -4 (preweaning), 0 (weaning), and then weekly for 4 weeks in the nursery. Blood and tissue samples were collected at d -4 (preweaning), 0 (weaning), 14, and 27-30 of the nursery for complete blood count (CBC) and tissue mineral concentration (Fe, Zn, Cu) analysis. All data were subjected to ANOVA by using the individual pig as the experimental unit. Pigs that received an additional iron injection 4 days before weaning had a greater ($P < 0.05$) ADG compared to control pigs at weeks 1, 2, and 3. The treatment pigs also had greater ($P \leq 0.01$) ADG for the overall nursery period, and the

overall experimental period including the 4 days preweaning. Consequently, the accumulation of improved growth by pigs that were administered an additional iron injection before weaning led to a heavier ($P < 0.001$) final BW (~1 kg) at 4 weeks in the nursery compared to control pigs. Treatment pigs had higher ($P < 0.001$) Hb, HCT, RBC, WBC, MCV and MCH values compared to the control pigs at weaning. The additional iron injected pigs continued to have higher ($P \leq 0.02$) Hb, MCV, and MCH values at d 14. However, by the end of the experiment, all CBC measures were similar between the control and treatment pigs. Similar to the blood measures, the iron concentration of the liver, spleen, heart, and kidney were all numerically greater for the added-injection pigs at weaning compared to the control pigs. The results of this experiment suggest that an additional iron injection administered 4 days before weaning may benefit overall growth performance as well as improve iron status in the blood and tissues at weaning.

Key Words: iron, iron injection, pigs, weaning, preweaning

5.2 Introduction

Modern swine production has undergone improvements in genetic potential leading to increased growth rates of nursing piglets. It is routine practice to supplement iron to newborn pigs shortly after birth as they have limited sources of iron. However, recent work has shown that the iron supplemented to piglets shortly after birth is not sufficient to maintain the iron status of the pig throughout the lactation period (see Chapter 4; Bhattarai and Nielsen, 2015; Perri et al., 2016) In addition, the faster-growing pigs are more susceptible to become iron deficient around weaning causing postweaning problems (Jolliff and Mahan., 2011). Morales et al. (2018) reported a decline in serum ferritin concentration on d 17 and 21 from the previous time on d 14 for pigs administered iron (200 mg iron) after birth indicating that an initial iron injection may only last 14 to 17 days. If so pigs that do grow faster and require more iron during lactation often are predisposed to an iron gap during the weaning transition. This is where the iron status has declined in the latter part of the nursing period, then consequently the decline becomes exacerbated by the weaning stress and low feed intake during the first few days postweaning.

A practical solution to compensate for the low iron status at weaning would be to consider an additional iron injection before weaning. Work by Urbaniak et al. (2017) reported larger pigs (> 6 kg) at weaning that received a second iron injection had greater growth performance than single injected pigs. Recent work at Kansas State (Williams et al., 2018b) revealed that at 21 and 35d post-partum pigs receiving an additional iron injection (administered at d 11) had higher hemoglobin concentration than pigs only receiving a single injection at processing (d 3). Estienne (2018) showed similar benefiting

growth results in the nursery period and increased hematocrit when injecting a second intramuscular injection of iron at weaning (Estienne and Clark-Deener, 2018).

Van Gorp et al. (2012) suggest that a nursing pig with a normal growth of 7 kg over a 28 d lactation period needs approximately 390 mg iron to last until a dietary source is available. Fredericks et al. (2018) reported that pigs classified at weaning with optimal hemoglobin levels (> 11 g/dL) had greater BW at 8 weeks postweaning compared to pigs under the optimal level. Therefore, it is believed that optimizing the iron status at weaning can have immense benefits in the nursery. Thus the objective of the present experiment was to evaluate the effects of administering an additional iron injection 4 days before weaning on growth performance, hematological status, and tissue mineral concentration pre and postweaning.

5.3 Experimental procedures

This experiment was conducted in environmentally controlled rooms at the University of Kentucky Swine Research Center under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

5.3.1 Animals, housing, management, and experimental design

A total of 136 crossbred pigs [82 barrows and 54 gilts; (Yorkshire x Landrace) x Large White] from 20 litters with an initial BW of 5.48 ± 1.08 kg were assigned to either a control or treatment group at around 14-20 days of age through a pairing scheme. At around 1 d of age, all pigs were subjected to normal farm processing procedures (tail docking, ear notching, and needle-teeth removal). At this same time, all piglets received a 150 mg iron intramuscular (IM) injection of iron dextran (Henry Schein Animal Health, Dublin, OH) in the right side of the neck. The pairing process selected pigs based on the

following: two pigs from the same litter, with the same sex, and a BW difference of < 0.41 kg. Within a given pair, one pig was assigned to the treatment group and the other pig to the control group. Pigs assigned to the treatment group received an additional 150 mg iron IM injection 4 days before weaning (14-20 days). In contrast, the pigs allotted to the control group received no additional injection at this time.

Pigs were weaned in two different groups to a nursery site 4 days following the additional iron injection of the treatment pigs (18-24 days). The weaning groups were based on the farrowing schedule of selected litters. In the nursery, pigs were allotted to pens based on BW, treatment, and sex. Pens consisted of 3 to 5 pigs per pen and were equalized between treatments. The experiment continued through 27-30 days in the nursery.

All nursery pens (1.22 m × 1.22 m) were elevated off of the ground in an environmentally-controlled room and had plastic coated wire flooring. All pens were equipped with a three-hole plastic feeder and a nipple waterer. Pigs had ad libitum access to water and feed for the duration of the experiment. Both the control and treatment pigs received the same nursery diets.

A total of 8 pigs (4 pigs per treatment) were selected for sacrifice on days -4 (pre-wean), 0 (weaning), 14, and 28. All pigs used for tissue mineral determination in this experiment were barrows. The liver, spleen, kidney, and heart samples were collected from the sacrificed pigs.

5.3.2 Experimental diets

All pigs received common two-phase nursery diets that were formulated to meet or exceed NRC (2012) requirement estimates for pigs 7 to 25 kg (Table 5.1). Phase I and II diets were fed for 14 and 16 days respectively. Representative diet samples were collected at the time of diet mixing and stored at -20°C until further analysis. The trace mineral premix used in both phases supplied the following per kilogram of the diet: 50 mg of Mn as manganous sulfate, 100 mg of Fe as ferrous sulfate, 125 mg of Zn as zinc sulfate, 20 mg of Cu as copper sulfate, 0.35 mg of I as calcium iodate, and 0.30 mg of Se as sodium selenite.

Phase I and II diets were analyzed for trace mineral concentration (Fe, Zn, and Cu). The analyzed trace mineral composition of Phase I diets was 220 ppm Fe, 138 ppm Zn, and 19 ppm Cu. For Phase II diets, the analyzed mineral composition was 247 ppm Fe, 140 ppm Zn, and 19 ppm Cu. Both diets were also analyzed for Ca and P content, for Phase I the Ca and P concentration was 0.79% and 0.62%, respectively. For the Phase II diets Ca and P were 0.75% and 0.66%, respectively.

Table 5.1. Composition of nursery diets (as-fed basis)

Item	Phase I	Phase II
Ingredient, %		
Corn	50.50	57.41
Soybean meal, 48% CP	28.50	32.50
Grease, choice white	2.00	2.00
Fish meal (Menhaden)	5.00	0.00
Spray-dried animal plasma	2.00	0.00
Whey dried	10.00	5.00
L-Lysine•HCl	0.07	0.24
DL-Methionine	0.05	0.13
L-Threonine	0.07	0.14
Dicalcium phosphate	0.33	0.97
Limestone	0.77	0.90
Salt	0.50	0.50
Trace mineral premix[1]	0.15	0.15
Vitamin premix[2]	0.04	0.04
Santoquin[3]	0.02	0.02
Total	100.00	100.00
Calculated Composition		
Metabolizable energy, kcal/kg	3423.00	3404.00
Crude protein, %	23.79	21.22
SID Lysine, %	1.35	1.23
Calcium, %	0.80	0.70
STTD Phosphorus, %	0.36	0.29

[1] Supplied the following per kilogram of diets: 50 mg of Mn as manganous sulfate, 100 mg of Fe as ferrous sulfate, 125 mg of Zn as zinc sulfate, 20 mg of Cu as copper sulfate, 0.35 mg of I as calcium iodate, and 0.30 mg of Se as sodium selenite.

[2] Supplied the following per kilogram of diets: 4,245 IU of vitamin A; 1,062 IU of vitamin D3; 28.3 IU of vitamin E; 3.2 IU of vitamin K; 0.012 mg of vitamin B12; 9.45 mg of pantothenic acid; 0.104 mg of biotin; 0.076 mg of folic acid; 18.81 mg of niacin; 1.89 mg of vitamin B6; and 0.52 mg of thiamin.

[3] Santoquin (Monsanto, St. Louis, MO) supplied 130 mg/kg ethoxyquin to the diets.

5.3.3 Data and sample collection

5.3.3.1 Feed collection

Representative samples of corn, soybean meal, and mixed feed were collected in the feed mill for both phases of the experimental diets. Feed samples were stored at -20°C until analyzed.

5.3.3.2 Growth performance and blood collection

Pigs were weighed at d -4 (preweaning) to be allotted to the control or treatment group. Pigs and feeders were also weighed at d 0 (weaning), and then weekly for 4 weeks in the nursery to determine ADG, ADFI, and F:G. Blood samples were collected from each pig through jugular venipuncture at d -4, 0, 14, and 27 or 30 of the nursery. The final blood collection was sampled on either d 27 or 30 of the nursery depending on the weaning time for the pair. Blood was collected in 3 mL vacutainer tubes coated with K_2EDTA (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood samples were immediately placed on ice and transported to the University of Kentucky Veterinary Diagnostic Laboratory (UKVDL) for complete blood count (CBC) analysis.

5.3.3.3 Tissue collection

A total of 32 barrows over the course of the experiment were sacrificed for tissue mineral concentration. Representative pigs (4 pigs per treatment) of the control and treatment groups were euthanized on d -4, 0, 14, and 27 or 30 of the nursery by injection of sodium pentobarbital (SOCUMB, Henry Schein Animal Health, Dublin, OH). The final tissue collection occurred on d 27 or 30 depending on the weaning time for the pair. Following euthanasia, pigs were dissected for tissue (liver, spleen, both kidneys, and heart) collection, then weighed, and stored at -20°C until further analysis.

5.3.4 Sample processing and laboratory analysis

5.3.4.1 Experimental diet measures

For micro-mineral (Zn, Fe, and Cu) and calcium analysis, feed samples were first digested using a microwave digester (MARS 6, CEM Cooperation, Matthews, NC), then analyzed for the minerals by flame atomic absorption spectrometry (Thermoelemental, SOLAAR Mf; Thermo Electron Corp., Verona, WI). Phosphorus content was analyzed using a gravimetric determination method (modification of method 968.08; AOAC, 1990).

5.3.4.2 Blood and tissue measures

Whole blood samples were transported to the UKVDL where they were analyzed for a complete blood count (CBC) using a hematological analyzer (Forcyte Veterinary Hematology Analyzer, Oxford Science, Oxford, CT). Before analysis, all blood samples were thoroughly mixed and brought to room temperature. The CBC analysis provided hemoglobin concentration (Hb), hematocrit (HCT), red blood cell count (RBC), white blood cell count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Tissue samples were placed through a kitchen-grade meat grinder (The butcher shop premium, KRUPS USA, Parsippany, NJ) to provide a homogenous tissue sample. After samples were ground and mixed, around 1-2 g of tissue was digested with nitric acid in a pressurized microwave digester (MARS 6 CEM, Matthews, NC) according to recommendations set by the manufacturer, and appropriately diluted. Diluted samples were analyzed for trace mineral composition (Zn, Fe, and Cu) by flame atomic absorption spectrophotometry (Thermoelemental, SOLAAR M5; Thermo Electron Corp., Verona,

WI). Dry matter (DM) was determined for all tissue samples by placing 2-3 g of ground sample into a gravity convection drying oven at 107°C for approximately 24 hours and then weighed again to observe the moisture content lost.

5.3.5 Statistical analysis

Prior to analyses, all data were evaluated to identify any potential statistical outliers detected by Grubb's test outlier calculator (Graphpad Software, San Diego, CA). All data were subjected to ANOVA by using the GLM procedure in SAS (Statistical Analysis System, Cary, NC). The individual pig was the experimental unit for individual BW and ADG, CBC measures, and tissue mineral concentrations and the results are reported as least squares means. Treatment least squares means were calculated using the LSMEANS option and of SAS. The level of significance was determined by a P-value of < 0.05. The data of individual BW and ADG, as well as CBC, were analyzed by the model:

$Y_{ijk} = \mu + T_i + sex_j + pair_k + (T \times sex)_{ij} + e_{ijk}$, where

Y = the response variables (BW, ADG; Hb, HCT, RBC, WBC, MCV, MCH, MCHC)

μ = a constant common to all observations

T_i = the treatment

sex_j = sex of the pig

$pair_k$ = the pair

$(T \times sex)_{ij}$ = the treatment × sex interaction

e_{ijk} = the error term of the model

The data for pen performance (pen ADG, ADFI, and F:G) were analyzed using the pen as the experimental unit and by the model:

$Y_i = \mu + T_i + sex_j + (T \times sex)_{ij} + e_{ij}$, where

Y = the response variables (ADG, ADFI, F:G)

μ = a constant common to all observations

T_i = the treatment

sex_j = the sex of pen

$(T \times sex)_{ij}$ = the treatment × sex interaction

e_{ij} = the error term of the model

The data for tissue mineral concentration was analyzed by the model:

$Y_{ij} = \mu + T_i + pair_j + e_{ij}$, where

Y = the response variables (tissue mineral concentration)

μ = a constant common to all observations

T_i = the treatment

$pair_j$ = the pair

e_{ij} = the error term of the model

5.4 Results

5.4.1 Growth performance

The results for growth performance on an individual basis are presented in Table 5.2. Before the additional iron injection administered to the treatment group, the control and treatment groups had an initial BW difference of 0.03 kg, which was still present at weaning (0.02 kg). Once weaned, pigs from the treatment group had greater ADG ($P < 0.05$) during weeks 1, 2, and 3. The ADG was also higher ($P \leq 0.01$) for pigs receiving the additional iron injection during Phase I, Phase II, and overall nursery period. Altogether the treated pigs had an increased ($P < 0.001$) ADG for the entire experimental period (pre-injection to nursery week 4) leading to a heavier ($P < 0.001$) final BW of approximately 1 kg.

Growth performance data using the nursery pen as the experimental unit is presented in Table 5.3. Values differ somewhat from individual data in Table 5.2, but this presentation is done to assess the ADFI and F:G. The added-injection treatment had an increased ($P = 0.03$) ADG for Phase I. The ADFI for Phase I, weeks 2, and 3 tended to be higher ($P = 0.09$, 0.09, and 0.08; respectively) for the treatment group. However, feed efficiency was not different between the control and treatment groups. There was no treatment by sex interactions ($P \geq 0.10$) observed for growth performance, however, the barrows did grow faster compared to the gilts leading to some sex effects for growth performance observed throughout the trial.

Table 5.2. Effects of an additional iron injection on individual growth performance[1,2]

Variable	Control	Added-injection	SEM	P-value		
				Treatment	Sex	TRT*Sex
BW, kg						
Pre-injection	5.43	5.46	0.02	0.21	0.12	0.79
Weaning	6.49	6.51	0.03	0.73	0.05	0.96
Nursery week 1	8.22	8.38	0.05	0.03	0.28	0.89
Nursery week 2	11.50	11.96	0.09	<0.001	0.52	0.88
Nursery week 3	16.49	17.29	0.14	<0.001	0.08	0.45
Nursery week 4	21.65	22.61	0.19	<0.001	0.05	0.38
ADG, g						
Pre-wean	266.1	261.2	5.74	0.55	0.14	0.87
Nursery week 1	246.5	268.4	6.86	0.03	0.01	0.90
Nursery week 2	469.1	510.9	7.67	<0.001	0.96	0.91
Nursery week 3	661.2	704.6	11.60	0.01	<0.01	0.17
Nursery week 4	737.3	760.4	11.10	0.14	0.11	0.44
Phase I (wk 1-2)	357.8	389.6	5.62	<0.001	0.12	0.88
Phase II (wk 3-4)	697.8	730.8	9.23	0.01	0.01	0.21
Overall Nursery[3]	530.5	563.0	6.24	<0.001	0.13	0.32
Overall Experiment[4]	498.0	526.2	5.57	<0.001	0.09	0.36

[1]Treatment means are representative of 60 pigs per treatment; reduced to 56 pigs/treatment after Week 2.
[2]Weaning was at 18 to 24 days of age.
[3]Overall nursery represents nursery week 1 through nursery Week 4.
[4]Overall experiment represents pre-weaning through nursery Week 4.

Table 5.3. Effects of an additional iron injection on pen growth performance in the nursery[1]

Variable	Control	Added-injection	SEM	P-value		
				Treatment	Sex	TRT*Sex
ADG, g						
Nursery week 1	244.3	270.0	11.34	0.12	0.06	0.82
Nursery week 2	471.6	513.4	14.73	0.06	0.91	0.98
Nursery week 3	663.9	708.4	18.59	0.10	0.08	0.33
Nursery week 4	735.6	756.3	17.96	0.43	0.24	0.73
Phase I (wk 1-2)	358.3	390.0	10.46	0.03	0.27	0.88
Phase II (wk 3-4)	698.6	730.6	15.37	0.15	0.08	0.46
Overall Nursery[2]	530.6	561.5	13.61	0.11	0.52	0.65
ADFI, g						
Nursery week 1	295.0	321.3	14.92	0.22	0.43	0.94
Nursery week 2	598.7	646.8	19.39	0.09	0.65	0.46
Nursery week 3	956.3	1026.4	27.07	0.08	0.35	0.66
Nursery week 4	1169.8	1213.9	36.55	0.40	0.30	0.95
Phase I (wk 1-2)	446.8	484.0	14.92	0.09	0.49	0.66
Phase II (wk 3-4)	1063.0	1120.2	30.38	0.20	0.30	0.82
Overall Nursery[2]	753.1	803.1	22.22	0.13	0.64	0.94
F:G						
Nursery week 1	1.21	1.19	0.03	0.64	0.08	0.99
Nursery week 2	1.27	1.26	0.02	0.71	0.46	0.10
Nursery week 3	1.45	1.45	0.02	0.98	0.14	0.39
Nursery week 4	1.67	1.66	0.02	0.64	0.66	0.37
Phase I (wk 1-2)	1.25	1.23	0.02	0.64	0.55	0.25
Phase II (wk 3-4)	1.52	1.53	0.02	0.72	0.43	0.49
Overall Nursery[2]	1.42	1.42	0.01	0.79	0.70	0.21

[1]Treatment means are representative of 17 pens per treatment; reduced to 15 pens/treatment after Week 2 when select pigs were euthanized for tissue collection.
[2]Overall nursery represents nursery week 1 through nursery week 4.

5.4.2 Hematological measures

Tables 5.4 to 5.10 represent the CBC data using the individual pig as the experimental unit. Pigs from both the control and treatment groups had a similar CBC profile during preweaning sampling. At weaning, the treatment pigs had higher ($P < 0.001$) Hb, HCT, RBC, WBC, MCV and MCH values. Interestingly, also at weaning the control group had a numerical decrease in Hb, HCT, WBC, MCV, and MCH content compared to the previous sampling at d -4. Hemoglobin concentration continued to be higher ($P < 0.02$) in the treatment group at d 14 sampling but not at d 27-30. Furthermore, at d 14, the MCV, MCH, and MCHC content was higher ($P \leq 0.02$) in pigs administered the additional iron injection. By the end of the experiment (d 27-30), there were no differences in the CBC profiles for both groups of pigs. There was no treatment by sex interactions observed for any CBC measures, however, the barrows did exhibit some increases in blood measures after weaning (d 14 and d 27-30) which caused a sex effect for certain measures (RBC, MCV, and MCHC).

Table 5.4. Effects of an additional iron injection on hemoglobin concentration (Hb, g/dL)[1]

				P-value		
Time	Control	Added-injection	SEM	Treatment	Sex	TRT*Sex
Preweaning	10.9	10.7	0.11	0.31	0.69	0.56
Weaning	10.4	12.0	0.12	<.0001	0.49	0.46
d 14	11.5	11.9	0.09	0.01	0.08	0.55
d 27-30[2]	12.8	12.8	0.09	0.84	0.07	0.74

[1]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[2]Final blood collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.5. Effects of an additional iron injection on hematocrit (HCT, %)[1]

				P-value		
Time	Control	Added-injection	SEM	Treatment	Sex	TRT*Sex
Preweaning	33.1	32.8	0.36	0.51	0.44	0.31
Weaning	31.6	36.5	0.37	<.0001	0.52	0.22
d 14	35.1	35.5	0.28	0.32	0.23	0.60
d 27-30[2]	38.1	38.1	0.29	0.97	0.89	0.42

[1]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[2]Final blood collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.6. Effects of an additional iron injection on red blood cell count (RBC, $10^6/\mu L$)[1]

				P-value		
Time	Control	Added-injection	SEM	Treatment	Sex	TRT*Sex
Preweaning	5.64	5.68	0.06	0.64	0.55	0.17
Weaning	5.87	6.23	0.07	<0.001	0.59	0.22
d 14	6.40	6.28	0.05	0.10	0.01	0.97
d 27-30[2]	6.72	6.61	0.06	0.17	0.26	0.61

[1]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[2]Final blood collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.7. Effects of an additional iron injection on white blood cell count (WBC, $10^3/\mu L$)[1]

				P-value		
Time	Control	Added-injection	SEM	Treatment	Sex	TRT*Sex
Preweaning	7.98	7.97	0.26	0.97	0.64	0.83
Weaning	7.72	9.27	0.32	<0.01	0.58	0.75
d 14	14.40	14.65	0.38	0.65	0.19	0.46
d 27-30[2]	13.23	12.75	0.46	0.45	0.91	1.00

[1]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[2]Final blood collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.8. Effects of an additional iron injection on mean corpuscular volume (MCV, fL)[1]

				P-value		
Time	Control	Added-injection	SEM	Treatment	Sex	TRT*Sex
Preweaning	58.8	57.8	0.45	0.12	0.86	0.54
Weaning	53.9	58.8	0.41	<.0001	0.79	0.89
d 14	55.0	56.8	0.34	<0.001	0.05	0.53
d 27-30[2]	56.9	57.7	0.31	0.05	0.05	0.66

[1]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[2]Final blood collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.9. Effects of an additional iron injection on mean corpuscular hemoglobin (MCH, pg)[1]

				P-value		
Time	Control	Added-injection	SEM	Treatment	Sex	TRT*Sex
Preweaning	19.4	18.9	0.17	0.09	0.77	0.27
Weaning	17.7	19.4	0.16	<.0001	0.74	0.46
d 14	18.1	19.0	0.12	<.0001	0.26	0.43
d 27-30[2]	19.1	19.4	0.14	0.17	0.54	0.92

[1]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[2]Final blood collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.10. Effects of an additional iron injection on mean corpuscular hemoglobin concentration (MCHC, g/dL)[1]

				P-value		
Time	Control	Added-injection	SEM	Treatment	Sex	TRT*Sex
Preweaning	32.9	32.8	0.12	0.38	0.27	0.15
Weaning	32.9	32.9	0.14	0.89	0.96	0.27
d 14	32.8	33.4	0.12	<0.01	0.24	0.85
d 27-30[2]	33.6	33.6	0.17	0.88	<0.01	0.53

[1]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[2]Final blood collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

5.4.3 Tissue measures

Liver, spleen, heart, and kidney samples were analyzed for mineral content from pigs at preweaning, weaning, and d 14 and d 27-30 of the nursery and presented as a DM basis. The average DM content for liver, spleen, heart, and kidney was 23.3, 20.0, 20.3, and 16.9 % respectively, throughout the experiment. In the control pigs, the liver iron content (Table 5.11) numerically decreased from the preweaning sample to weaning while the liver iron content of pigs that received the additional iron injection prior to weaning was much higher (P = 0.02) compared to the control pigs. However, by d 14 there was only a nonsignificant numerical increase in liver iron content for the added-injection group which became essentially equal at d 27-30. Liver Zn and Cu content did not seem to be impacted by the additional iron injection as both groups were similar at all periods of the experiment. However, there was a drastic decline in liver Cu for both treatments from weaning to the end of the experiment.

The iron content of spleen (Table 5.12) tended to be higher in added-injection pigs compared to the control pigs at weaning, but not at d 14, and d 27-30 of the nursery. The spleen iron content was 5 to 6 times higher at weaning than at preweaning and maintained that increase for the rest of the study. Similar to liver iron content, the heart iron content in the control pigs was numerically lower at weaning compared to preweaning. Also, similar to the liver and spleen, the iron content of the heart (Table 5.13) was numerically greater at weaning, d 14, and d 27-30 for the added-injection pigs. Observed once again, the control pigs had a decreased kidney iron content from preweaning to weaning compared to the added-injection pigs which had a numerical increase (Table 5.14). Interestingly the control pigs had a greater (P = 0.03) heart copper

content at d 14 than those of the pigs injected before weaning (Table 5.13). More so, the heart copper concentration remained relatively constant from preweaning to the end of the experiment compared to the liver and spleen, where there was at least a 50% decrease in copper concentration from weaning to d 27-30.

Table 5.11. Effects of an additional iron injection on liver mineral concentration (DM basis, mg/kg)[1,2]

Variable	Control	Added-injection	SEM	P-value
Preweaning				
Fe	495.1			
Zn	305.3			
Cu	373.3			
Weaning				
Fe	274.4	809.2	125.88	0.02
Zn	346.0	316.2	63.11	0.75
Cu	392.3	393.9	53.94	0.99
d 14				
Fe	476.5	536.4	36.26	0.29
Zn	300.4	309.0	23.64	0.81
Cu	103.4	118.5	28.88	0.69
d 27-30[3]				
Fe	598.9	600.4	41.29	0.98
Zn	427.8	412.1	56.53	0.85
Cu	19.1	8.3	3.99	0.10

[1]Means at preweaning represent 8 pigs; treatment means at weaning, d 14 and 27-30 represent 4 pigs per treatment.
[2]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[3]Final tissue collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.12. Effects of an additional iron injection on spleen mineral concentration (DM basis, mg/kg)[1,2]

Variable	Control	Added-injection	SEM	P-value
Preweaning				
Fe		151.1		
Zn		60.2		
Cu		4.3		
Weaning				
Fe	750.6	1024.2	100.01	0.10
Zn	88.5	90.1	8.90	0.91
Cu	6.7	4.8	0.93	0.19
d 14				
Fe	802.6	885.0	82.92	0.51
Zn	159.0	150.5	8.39	0.50
Cu	3.6	3.6	1.04	0.97
d 27-30[3]				
Fe	711.1	847.1	117.15	0.44
Zn	160.2	152.1	11.52	0.63
Cu	2.3	2.1	0.09	0.67

[1]Means at preweaning represent 8 pigs; treatment means at weaning, d 14 and 27-30 represent 4 pigs per treatment.
[2]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[3]Final tissue collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.13. Effects of an additional iron injection on heart mineral concentration (DM basis, mg/kg)[1,2]

Variable	Control	Added-injection	SEM	P-value
Preweaning				
Fe		234.6		
Zn		61.4		
Cu		16.5		
Weaning				
Fe	170.1	270.4	37.62	0.11
Zn	63.7	79.9	10.57	0.32
Cu	15.4	19.1	2.88	0.40
d 14				
Fe	251.2	266.8	30.72	0.73
Zn	62.4	58.8	2.08	0.26
Cu	16.0	13.8	0.53	0.03
d 27-30[3]				
Fe	202.4	240.2	23.15	0.29
Zn	63.5	59.8	1.52	0.14
Cu	16.0	15.3	1.09	0.66

[1]Means at preweaning represent 8 pigs; treatment means at weaning, d 14 and 27-30 represent 4 pigs per treatment.
[2]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[3]Final tissue collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

Table 5.14. Effects of an additional iron injection on kidney mineral concentration (DM basis, mg/kg)[1,2]

Variable	Control	Added-injection	SEM	P-value
Preweaning				
Fe	179.8			
Zn	73.1			
Cu	33.5			
Weaning				
Fe	138.8	252.9	45.19	0.12
Zn	77.3	79.2	1.38	0.36
Cu	35.9	38.6	3.56	0.69
d 14				
Fe	247.9	396.7	71.57	0.19
Zn	81.0	82.1	2.52	0.78
Cu	32.8	34.3	2.77	0.72
d 27-30[3]				
Fe	368.5	367.3	29.10	0.98
Zn	94.5	82.8	5.73	0.20
Cu	41.5	32.3	3.73	0.13

[1]Means at preweaning represent 8 pigs; treatment means at weaning, d 14 and 27-30 represent 4 pigs per treatment.
[2]Preweaning was 4 d before weaning, weaning was at 18 to 24 days of age.
[3]Final tissue collection was either d 27 or 30 depending on the weaning time of the pair and due to experimental scheduling issues.

5.5 Discussion

5.5.1 Growth performance

The addition of a second iron injection (150 mg iron) administered to pigs 4 days before they were weaned resulted in an increased ADG throughout the experimental period. The increase in ADG is likely a result of the higher ADFI that the added-injection pigs demonstrated. Jolliff and Mahan (2011) reported a greater ADFI ($P < 0.05$) from 7 to 21d-postweaning in pigs injected with an additional iron injection 6 days before weaning (d 17). Kamphues et al. (1982) reported that pigs administered a second iron injection one week prior to weaning had an increase in daily BW gain (380 g vs. 362 g) through three weeks in the nursery agreeing with the current findings of a 33 g increase during a 4 week nursery period. However, in contrast to the current experiment, Williams et al. (2018) found no effect on growth performance in pigs given a second iron injection. Notably, however, Williams et al. (2018) administered the second iron injection on d 11 and weaned pigs on d 21 compared to the current experiment where the second injection was administered at 14-20 days (d -4). Thus the time of the second injection may explain the differences observed in growth performance.

The second iron injection will not be as efficient if the pig has substantial amounts of body iron (less of a demand); closer to weaning or later in lactation the iron status of the pig declines which increases the demand for more iron. Holter et al. (1991) and Jolliff and Mahan (2011) reported a reduction in hematological measures as early as 17 days for pigs that were administered an iron supplement at birth (180 and 200 mg iron, respectively). The reduction in iron status stated previously, and considering a standard

weaning age in the United States of ~21 days, the concept of a second iron injection administered 4 days before weaning seems appropriate.

5.5.2 Hematological and tissue measures

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

In the current experiment, administering an additional iron injection 4 days before weaning resulted in increased Hb, HCT, RBC, WBC, MCV, and MCH at weaning. The improvement in hematological measures at weaning in the current experiment can be explained from the additional iron given to the pigs. These findings are in agreement with

other similar work in which a second iron injection improved hematological parameters (Haugegaard et al., 2008; Williams et al., 2018). It is proposed that an intramuscular injection of iron dextran is absorbed by the body relatively fast through the reticuloendothelial system due to the phagocytes in the liver, spleen, and bone marrow (Danielson, 2004). In regards to the rapid increase in hematological measures as early as 4 days after the additional iron injection in the current experiment, Pu et al. (2018) reported observations of iron accumulation in the liver of pigs as early as 5 days after injection. The previously stated literature is also in agreement with the current findings, showing that liver iron concentration was elevated 4 days after the second iron injection. Interestingly, a normal blood hemoglobin concentration, but reduced liver iron concentration can indicate the beginning of iron deficiency as hemoglobin synthesis will pull iron from body reserves (Conrad et al., 2002).

The liver, heart, and kidneys from pigs not receiving a second iron injection all had decreasing iron concentrations from preweaning to weaning. The reduction in tissue iron concentration may be from the body pulling iron from tissue reserves to support normal erythropoiesis and hemoglobin synthesis. Dallman (1986) suggests that of all the iron sites (hemoglobin, serum iron, etc.), the storage sites are the last to be depleted in an iron-deficient state.

Lastly, pigs injected with an additional iron supplement 4 days before weaning had a lower Cu concentration of the heart 2 weeks after weaning compared to pigs that were not given a second iron injection. The reduced heart copper concentration associated with pigs given more iron could be a result of an inhibitory interaction between iron and copper. Astrup and Lyso (1986) reported that high dietary iron had a negative effect that

reduced hepatic Cu levels. However, the results reported from Astrup and Lyso (1986) were from increased dietary ratios of iron to copper (20:1 and 40:1) which is different than the current experimental dietary ratio (11:1). Even though the dietary ratio is lower compared to previous literature, there could be an additive effect by dietary iron to copper as well as the iron injection administered before weaning. Alternatively, the observation of this effect is observed in only 1 of the 4 tissues and could simply be a type II statistical error.

5.6 Conclusion

The results of the present experiment demonstrated that an additional iron injection administered to pigs 4 days before weaning both increased iron status in the blood at weaning as well as promoted growth performance during the nursery. The second iron injection also led to improved tissue iron contents at weaning. These results and the literature reviewed suggest that the additional iron is beneficial to the iron status of pigs especially during the stressful time of weaning where there is a low feed intake. There are also possibilities of improved growth performance associated with a second iron injection, however, the timing of the second injection will be maximally efficient when administered at a time of iron decline from the initial injection. Therefore, future studies should aim to investigate the optimal time to administer a second iron injection.

CHAPTER 6.General discussion

Iron status of young pigs has been a topic of concern since the early transition from rearing pigs on pasture to raising them in modern confinement housing. The transition dates back to when McGowan and Crichton (1924) demonstrated that farrowing sows indoors on a concrete floor compared to pasture led to iron deficiency and anemia. Since this time, iron supplementation to piglets has been extensively researched as it is one of the largest nutritional deficiencies observed in modern pig production. Early research has indicated that pigs are born with very limited iron reserves and receive minimal iron from sow milk (Venn et al., 1947). It has also been proved that it is necessary to administer an iron injection early after birth to prevent iron-deficient anemia. The latest edition of the NRC (2012) suggests administering 100 to 200 mg Fe intramuscularly within the first few days after birth.

With continuous improvements in the modern swine industry like improved genetic selection, productivity is at an all-time high. Under greater production demands, certain nutrient requirements like iron change. There is a growing concern that iron supply provided at birth is not sufficient to meet the iron requirement of every pig until they transition to a feed source that offers adequate iron.

Jolliff and Mahan (2011), Bhattarai and Nielsen (2015), and Perri et al. (2016) all demonstrated that there were pigs within herds that were deemed iron deficient (Hb concentration < 11 g/dL) at weaning. Jolliff and Mahan (2011) demonstrated that as weaning BW increased the Hb concentration at weaning decreased. Later work by Bhattaria and Nielsen (2015) found similar results in which larger piglets tended to be at an increased risk of lower Hb concentration and iron status at weaning. Perri et al. (2016)

found that pigs with an anemic hemoglobin concentration (< 8 g/dL) at weaning were 0.8 kg lighter than other pigs. Gillespie (2019) suggests that occurrences of sub-optimal iron levels (Hb < 11 g/dL) of pigs at weaning has been estimated to cost the United States swine industry millions of dollars.

In the first experiment of the current study (Chapter 3), there was an incidence of 50% (60 pigs) that had Hb concentrations below 11 g/dL at weaning after receiving an iron injection at birth (Table 3.3). These results demonstrated that there is a population within the University of Kentucky swine herd that has a sub-optimal iron status at weaning in agreement with previous work assessing iron status at weaning. The occurrence of lower hemoglobin concentration at weaning was in relationship to increasing BW and BW gain (Figure 3.2 and 3.3; respectively). Thus these findings were similar to the earlier work reported by Jolliff and Mahan (2011), Bhattarai and Nielsen (2015), and Perri et al. (2016). The similarity of UK pigs to previous observations suggests the pigs are suitable as a model for research pertaining to piglet iron questions. However in the present study, in the postweaning period (21 and 35d) there was a minimal incidence of hemoglobin concentration below the critical point; even more so, there were positive relationships between Hb concentrations and BW at these times (Figure 3.4 and 3.5; respectively). These results differ from the results by Perri et al. (2016), where the iron-deficient incidence increased from weaning to 3 weeks postweaning. Thus, there must be differences across herds or feeding practices that account for the differences. It was likely that the greater incidence observed in the work by Perri et al. (2016) was due to the high inclusion of zinc in the nursery diets.

With the realization that some pigs within the University of Kentucky swine herd demonstrate low iron status at weaning, the question of how the time course of hematological status changes during lactation and weaning for pigs receiving an iron injection at birth arose as well as whether there is an impact on growth or tissue mineral concentration. Therefore the time course of the blood profile and for pigs was evaluated during the pre and postweaning periods after receiving various amounts of iron (Chapter 4). During the second experiment growth and blood CBC were measured at many periods' pre and postweaning. Pigs that did not receive an iron injection at birth had lower ADG by the first week which led to a lower final BW on d 52. Growth during the present experiment was observed mainly at weeks 3, 4, and 5 for pigs with increasing injectable iron (0, 50, 100, 200, and 300). The improvement in growth observed at these times may be due to the declining MCV and MCH values most noticeably at d 17 to 22, which are lower than the initial values at birth for pigs with a lower iron dose. Holter et al. (1991) also demonstrated a decline in MCV and MCH at 17 and 21 days that surpassed initial values. Week 3 in the current experiment was also the time of weaning, which could be crucial to postweaning performance previously illustrated by Fredericks et al. (2018) where pigs with an improved iron status (Hb > 11 g/dL) at weaning had greater growth in subsequent periods.

Pigs that did not receive an iron injection had the lowest CBC measures and tissue iron concentrations through d 38 of the experiment indicating that they were in an anemic state. However, by d 52, these pigs seem to recover as CBC measurements are similar to other treatments. There was a similar pattern observed for CBC response measures that were exhibited when iron dosage increased, this pattern was clear early in the experiment

(d 3) and continued to d 38. Afterward, on d 52 there were no differences between treatments. The iron concentration of all tissues (liver, spleen, heart, and kidneys) were greater ($P \leq 0.01$) at weaning with increasing iron dosage. Interestingly, at weaning and d 38, the absolute and relative heart weight was higher ($P \leq 0.02$) for pigs receiving no iron injection. These results suggest that pigs receiving no iron experienced cardiac hypertrophy, where the heart accumulates extra muscle from operating with a more vigorous output (Dallman, 1986). The cardiac hypertrophy observed for the 0 mg iron treatment is supported by the low CBC measures for this group. Overall the 300 mg iron treatment may provide an advantageous supply of iron as this treatment had a consistency for higher CBC and tissue mineral concentrations.

Following the previous experiment (Chapter 4), there were additional questions that came about regarding whether improving iron status at weaning could lead to improved nursery performance. Thus, the effects of an additional iron injection administered 4 days before weaning on nursery growth performance, CBC, and tissue mineral concentration was assessed (Chapter 5). After receiving an additional iron injection before weaning, the pigs from the treatment group had improved ($P < 0.05$) ADG for nursery weeks 1, 2, and 3, as well as a numerical increased ADG during week 4 (Table 5.3). This accumulation of greater ADG led to a heavier ($P < 0.001$) final BW at 4 weeks in the nursery for the pigs administered the additional iron injection (Table 5.2). The improved growth performance for the treatment group could be due to the accumulation of numerically increased ADFI from weeks 1 through 4. In agreement with results from the current experiment, Kamphues et al. (1982) demonstrated that pigs given a second iron injection 1 week prior to weaning had improved daily gains (~18 g/d) through 3 weeks in the nursery. Also,

somewhat similar to the current findings were results reported by Jolliff and Mahan (2011) indicating greater ADFI from 7 to 21d-postweaning for pigs that received an additional iron injection 6 days before weaning although unlike the current experiment they found no differences in growth.

Also in the current experiment, pigs injected with a second iron injection resulted in greater CBC measures (Hb, HCT, RBC, WBC, MCV, and MCH) at weaning. These findings can simply be explained by the additional iron the pigs received. Additionally, iron content was greater in the liver, spleen, heart, and kidneys at weaning for the treatment pigs. These results are in agreement with findings by Pu et al. (2018), where they found iron accumulation in the liver as early as 5 days after injection concluding that iron can be absorbed and deposited in tissues rather quickly. These results indicate that optimizing iron at weaning by administering an additional iron injection, may be beneficial to growth, hematological status, and tissue mineral concentration at weaning and in the subsequent nursery period.

In summation, iron is an essential mineral to pigs. There were many positive effects seen with either increasing initial iron injection dosage or supplementing a second iron injection before weaning. However, an initial iron injection may not be adequate to suffice all pigs with their respective iron requirements by weaning. Therefore, further studies looking at supplementing additional iron during the lactation period and assessing the economic impact (return on investment, ROI) of the second iron injection may be beneficial to pork producers.

APPENDICES

Appendix 1. Effects of increasing iron injection dosage on the cumulative change of individual CBC measures

Table A.1. Effects of iron injection dosage on cumulative hemoglobin concentration (Hb, g/dL) change[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
0 to 1 d	-0.8	-0.7	-0.7	-0.3	-0.1	0.25	0.02	0.77
0 to 2 d	-1.4	-0.8	-1.0	-0.8	-0.8	0.25	0.12	0.37
0 to 3 d	-1.8	-0.8	-0.5	-0.5	-0.4	0.24	<0.001	0.02
0 to 4 d	-2.2	-0.7	-0.2	-0.3	-0.2	0.29	<.0001	<0.001
0 to 6 d	-2.8	-0.2	1.0	1.2	1.2	0.33	<.0001	<.0001
0 to 8 d	-3.3	-0.6	1.0	1.4	1.6	0.37	<.0001	<.0001
0 to 11 d	-3.6	-0.8	1.1	2.4	2.6	0.36	<.0001	<.0001
0 to 14 d	-3.8	-1.1	0.7	2.8	3.4	0.42	<.0001	<.0001
0 to 17 d	-4.0	-1.1	0.8	2.9	3.8	0.48	<.0001	<0.001
0 to 22 d	-4.1	-1.2	0.5	2.4	3.8	0.57	<.0001	<0.01
0 to 23 d	-4.1	-1.1	0.4	2.6	3.2	0.61	<.0001	<0.01
0 to 24 d	-4.1	-0.9	0.8	2.6	3.4	0.60	<.0001	<0.001
0 to 25 d	-4.0	-1.0	0.3	2.0	2.6	0.53	<.0001	<0.001
0 to 29 d	-1.3	1.7	2.6	2.7	2.4	0.51	<.0001	<.0001
0 to 38 d	1.7	2.8	3.0	2.3	1.9	0.46	0.63	0.04
0 to 52 d[2]	3.2	3.3	3.4	2.9	2.4	0.55	0.17	0.52

[1]Iron treatments were administered after d 0 blood sampling, and pigs were weaned on d 22.
[2]Treatment means reduced to 8 pigs per treatment.

Table A.2. Effects of iron injection dosage on cumulative hematocrit (HCT, %) change[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
0 to 1 d	-2.4	-2.2	-1.8	-1.1	-1.3	0.93	0.29	0.73
0 to 2 d	-4.0	-2.3	-1.8	-2.2	-1.9	0.91	0.19	0.28
0 to 3 d	-5.9	-2.4	-1.2	-1.7	-1.3	0.94	0.01	0.02
0 to 4 d	-6.2	-0.9	1.2	0.6	0.7	1.09	<0.001	<0.01
0 to 6 d	-8.0	1.5	6.2	5.4	6.2	1.14	<.0001	<.0001
0 to 8 d	-8.5	0.0	5.1	5.5	5.1	1.84	<.0001	<0.001
0 to 11 d	-11.2	-0.9	4.7	8.4	9.3	1.47	<.0001	<.0001
0 to 14 d	-10.5	-1.4	4.3	9.8	12.4	1.64	<.0001	<0.001
0 to 17 d	-10.7	-1.2	5.0	9.7	13.1	1.66	<.0001	<0.001
0 to 22 d	-10.0	-0.9	3.8	8.6	13.0	1.91	<.0001	0.01
0 to 23 d	-10.4	-0.5	3.8	9.8	10.9	2.04	<.0001	<0.01
0 to 24 d	-9.9	0.6	5.5	8.8	11.4	2.03	<.0001	<0.001
0 to 25 d	-9.4	0.3	4.3	8.6	9.6	1.78	<.0001	<0.001
0 to 29 d	-0.1	9.4	11.1	9.9	9.1	1.62	<0.01	<0.001
0 to 38 d	3.3	5.9	6.8	4.7	3.7	1.41	0.65	0.09
0 to 52 d[2]	6.1	6.3	7.0	5.9	4.9	1.78	0.50	0.58

[1]Iron treatments were administered after d 0 blood sampling, and pigs were weaned on d 22.
[2]Treatment means reduced to 8 pigs per treatment.

Table A.3. Effects of iron injection dosage on cumulative red blood cell count (RBC, 10^6/μL) change[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
0 to 1 d	-0.27	-0.21	-0.26	-0.10	-0.15	0.15	0.45	0.80
0 to 2 d	-0.44	-0.37	-0.43	-0.50	-0.45	0.13	0.69	0.87
0 to 3 d	-0.61	-0.50	-0.50	-0.56	-0.51	0.13	0.79	0.82
0 to 4 d	-0.59	-0.50	-0.38	-0.48	-0.47	0.14	0.65	0.46
0 to 6 d	-0.80	-0.12	0.11	-0.01	0.09	0.20	0.01	0.03
0 to 8 d	-0.82	0.06	0.27	0.21	0.33	0.23	<0.01	0.02
0 to 11 d	-1.05	0.30	0.65	0.75	0.91	0.27	<.0001	<0.01
0 to 14 d	-0.60	0.62	1.06	1.14	1.50	0.29	<.0001	0.01
0 to 17 d	-0.43	1.01	1.60	1.53	1.89	0.29	<.0001	<0.01
0 to 22 d	0.04	1.74	2.03	1.81	2.23	0.35	0.00	0.01
0 to 23 d	-0.08	1.88	2.10	2.09	1.95	0.37	<0.01	<0.01
0 to 24 d	0.04	2.12	2.46	1.90	2.06	0.35	<0.01	<0.001
0 to 25 d	-0.03	1.85	2.12	1.89	1.76	0.31	<0.01	<0.001
0 to 29 d	0.76	2.52	2.62	1.95	1.62	0.29	0.61	<0.001
0 to 38 d	0.71	1.31	1.46	0.99	0.68	0.22	0.30	0.02
0 to 52 d[2]	1.33	1.40	1.44	1.12	1.07	0.27	0.24	0.73

[1]Iron treatments were administered after d 0 blood sampling, and pigs were weaned on d 22.
[2]Treatment means reduced to 8 pigs per treatment.

Table A.4. Effects of iron injection dosage on cumulative white blood cell count (WBC, $10^3/\mu L$) change[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
0 to 1 d	-1.37	-3.00	-1.22	-2.13	-2.84	1.05	0.48	0.79
0 to 2 d	0.10	0.31	2.27	2.00	1.08	0.95	0.33	0.13
0 to 3 d	1.48	1.49	3.30	3.08	1.39	1.05	0.87	0.11
0 to 4 d	1.74	1.97	4.36	4.12	1.79	1.21	0.78	0.05
0 to 6 d	0.47	0.16	1.97	1.33	-0.43	1.17	0.66	0.17
0 to 8 d	-0.56	-1.45	-0.53	-1.52	-1.96	1.06	0.31	0.87
0 to 11 d	-1.55	-2.11	-1.36	-2.63	-2.02	0.99	0.59	0.79
0 to 14 d	-1.79	-3.23	-2.02	-3.69	-2.47	1.03	0.57	0.40
0 to 17 d	-1.45	-3.27	-2.30	-4.04	-2.53	1.22	0.46	0.27
0 to 22 d	-0.85	-3.66	-2.24	-3.76	-1.21	1.48	1.00	0.13
0 to 23 d	-0.61	-2.76	-0.62	-1.66	2.53	1.73	0.11	0.18
0 to 24 d	-1.14	-1.67	1.92	-0.63	4.86	2.27	0.04	0.52
0 to 25 d	-0.72	-1.59	-0.06	-1.80	3.66	1.73	0.07	0.14
0 to 29 d	0.14	0.47	3.58	0.06	5.69	2.24	0.11	0.59
0 to 38 d	4.51	4.06	3.35	0.94	3.55	1.52	0.32	0.21
0 to 52 d[2]	5.11	6.26	6.78	2.78	3.09	2.44	0.23	0.74

[1]Iron treatments were administered after d 0 blood sampling, and pigs were weaned on d 22.
[2]Treatment means reduced to 8 pigs per treatment.

Table A.5. Effects of iron injection dosage on mean corpuscular volume (MCV, fL) cumulative change[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
0 to 1 d	-1.6	-2.0	-0.6	-1.1	-0.7	0.66	0.24	0.71
0 to 2 d	-3.3	0.9	3.1	3.1	2.8	1.06	<0.001	<0.01
0 to 3 d	-6.1	3.5	6.7	6.1	6.0	1.45	<.0001	<.0001
0 to 4 d	-7.5	8.4	11.9	11.2	11.2	1.65	<.0001	<.0001
0 to 6 d	-8.8	7.9	15.5	14.4	14.6	2.27	<.0001	<.0001
0 to 8 d	-12.9	0.3	9.4	11.1	7.4	2.35	<.0001	<.0001
0 to 11 d	-13.3	-6.0	1.8	8.2	7.7	2.04	<.0001	<0.001
0 to 14 d	-19.7	-11.3	-4.6	5.5	5.6	1.73	<.0001	<.0001
0 to 17 d	-23.2	-15.6	-9.6	0.7	2.1	1.76	<.0001	<0.001
0 to 22 d	-27.8	-21.4	-15.8	-4.5	-1.6	1.27	<.0001	<0.001
0 to 23 d	-27.7	-21.8	-16.4	-5.3	-2.7	1.35	<.0001	<0.01
0 to 24 d	-28.4	-21.7	-16.6	-4.9	-3.0	1.45	<.0001	<0.001
0 to 25 d	-26.0	-20.2	-15.7	-5.3	-2.9	1.34	<.0001	<0.01
0 to 29 d	-11.5	-10.4	-8.7	-3.8	-2.1	1.51	<.0001	0.74
0 to 38 d	-3.0	-4.6	-4.7	-3.2	-1.3	1.04	0.05	0.07
0 to 52 d[2]	-5.5	-5.0	-4.6	-2.3	-3.5	0.99	0.03	0.28

[1]Iron treatments were administered after d 0 blood sampling, and pigs were weaned on d 22.
[2]Treatment means reduced to 8 pigs per treatment.

Table A.6. Effects of iron injection dosage on cumulative mean corpuscular hemoglobin (MCH, pg) change[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
0 to 1 d	-0.7	-0.6	-0.4	-0.2	0.8	0.61	0.07	0.53
0 to 2 d	-1.6	0.5	-0.1	0.9	0.8	0.61	0.02	0.14
0 to 3 d	-1.5	1.5	2.0	2.1	2.2	0.70	<0.01	0.01
0 to 4 d	-3.4	1.8	2.3	2.2	2.5	0.64	<.0001	<.0001
0 to 6 d	-3.5	0.8	2.2	2.6	2.6	0.92	<.0001	<0.01
0 to 8 d	-4.4	-1.3	1.1	2.2	0.9	1.14	<0.001	<0.01
0 to 11 d	-2.5	-3.1	-0.8	1.3	1.5	1.35	<0.01	0.66
0 to 14 d	-6.8	-5.2	-3.4	0.6	0.5	1.34	<.0001	0.18
0 to 17 d	-8.6	-6.7	-5.2	-0.6	-0.4	1.00	<.0001	0.11
0 to 22 d	-11.7	-8.7	-7.0	-2.5	-1.7	0.61	<.0001	<0.01
0 to 23 d	-11.6	-9.0	-7.4	-3.0	-1.7	0.63	<.0001	0.02
0 to 24 d	-11.9	-9.1	-7.6	-2.3	-1.8	0.68	<.0001	<0.01
0 to 25 d	-11.5	-8.8	-7.5	-3.3	-2.2	0.67	<.0001	0.02
0 to 29 d	-7.3	-5.9	-5.2	-2.5	-2.0	0.73	<.0001	0.24
0 to 38 d	0.2	0.0	-0.4	0.4	0.9	0.62	0.24	0.38
0 to 52 d[2]	0.3	0.5	0.3	0.7	0.0	0.58	0.70	0.50

[1]Iron treatments were administered after d 0 blood sampling, and pigs were weaned on d 22.
[2]Treatment means reduced to 8 pigs per treatment.

Table A.7. Effects of iron injection dosage on cumulative mean corpuscular hemoglobin concentration (MCHC, g/dL) change[1]

	Iron injection, mg Fe						Contrast	
Time	0	50	100	200	300	SEM	L	Q
No. of Pigs	10	10	10	10	10			
0 to 1 d	-0.3	0.2	-0.5	0.3	1.5	1.03	0.22	0.53
0 to 2 d	-0.8	0.2	-1.9	-0.4	-0.4	0.94	0.83	0.61
0 to 3 d	0.7	0.3	-0.6	-0.1	0.2	0.92	0.73	0.37
0 to 4 d	-1.5	-1.5	-2.6	-2.2	-1.8	0.84	0.76	0.54
0 to 6 d	-1.2	-2.7	-4.1	-3.0	-3.1	0.96	0.25	0.11
0 to 8 d	-0.7	-2.3	-3.1	-2.3	-2.5	1.06	0.35	0.27
0 to 11 d	2.5	-1.9	-2.3	-2.3	-1.6	1.50	0.10	0.04
0 to 14 d	-1.5	-2.6	-3.1	-2.0	-2.1	1.54	0.99	0.57
0 to 17 d	-2.6	-3.0	-3.6	-1.6	-1.8	1.10	0.26	0.68
0 to 22 d	-5.9	-3.5	-3.4	-1.8	-1.9	0.86	<0.001	0.09
0 to 23 d	-5.7	-4.0	-3.7	-2.2	-1.4	0.86	<0.001	0.42
0 to 24 d	-6.1	-4.2	-4.1	-1.3	-1.5	0.96	<.0001	0.18
0 to 25 d	-6.8	-4.5	-4.5	-2.7	-2.1	0.88	<0.001	0.28
0 to 29 d	-6.2	-4.5	-4.0	-2.2	-2.2	0.79	<0.001	0.13
0 to 38 d	1.8	2.6	2.0	2.3	1.9	0.77	0.95	0.66
0 to 52 d[2]	3.5	3.7	3.1	2.6	1.9	0.77	0.06	0.81

[1]Iron treatments were administered after d 0 blood sampling, and pigs were weaned on d 22.
[2]Treatment means reduced to 8 pigs per treatment.

Appendix 2. Effects of iron injection dosage on individual CBC measures during pre and postweaning

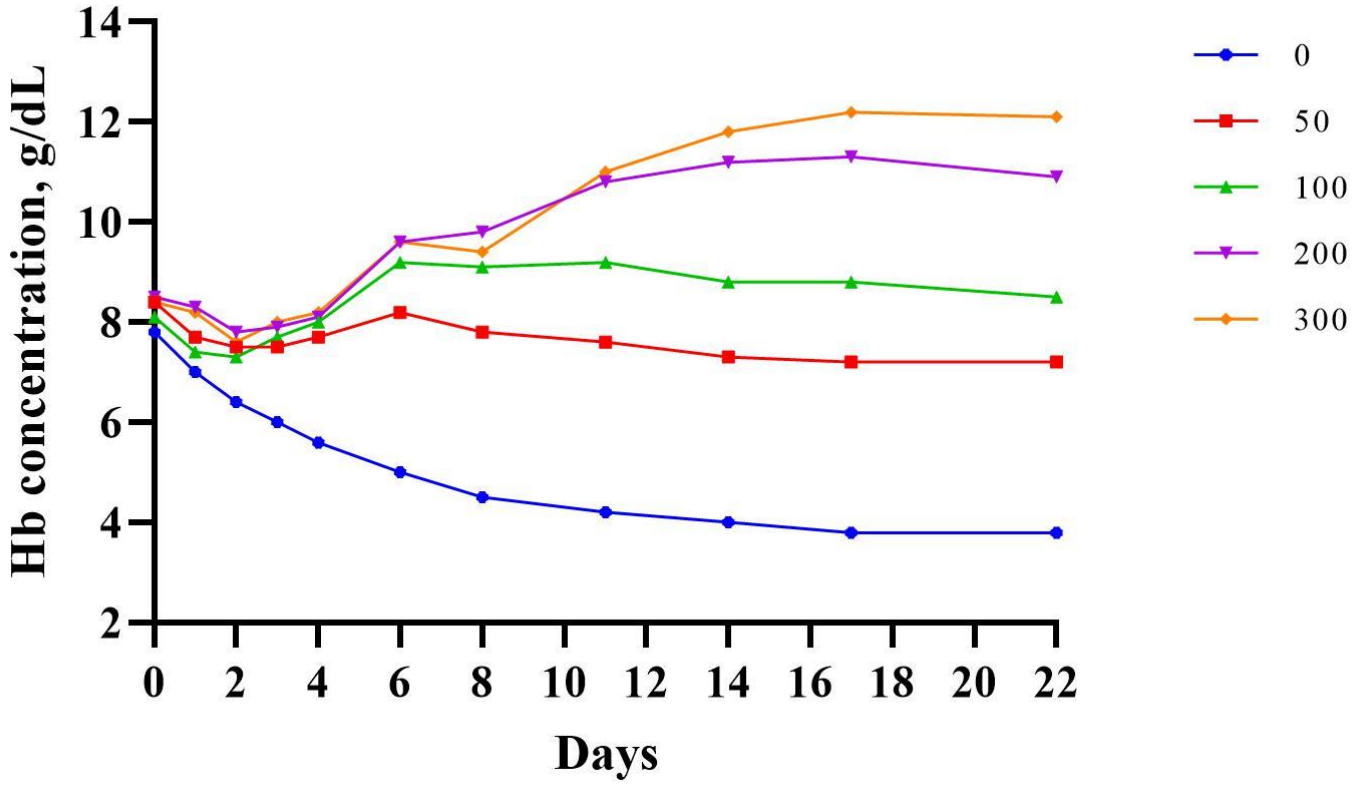

Figure A.1. Effects of iron injection dosage on hemoglobin concentration (Hb) during the preweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P = 0.0176$.

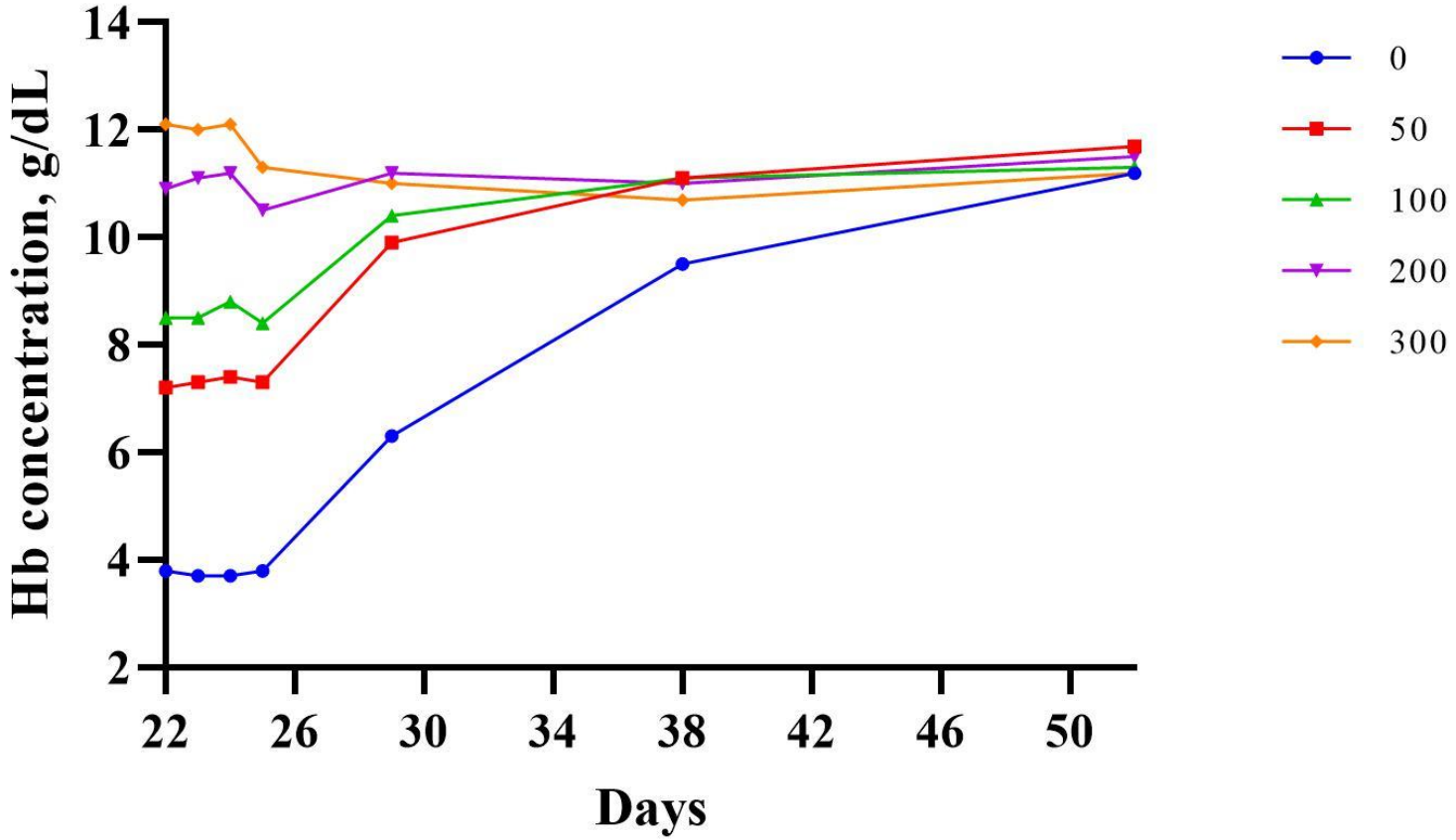

Figure A.2. Effects of iron injection dosage on hemoglobin concentration (Hb) during the postweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$.

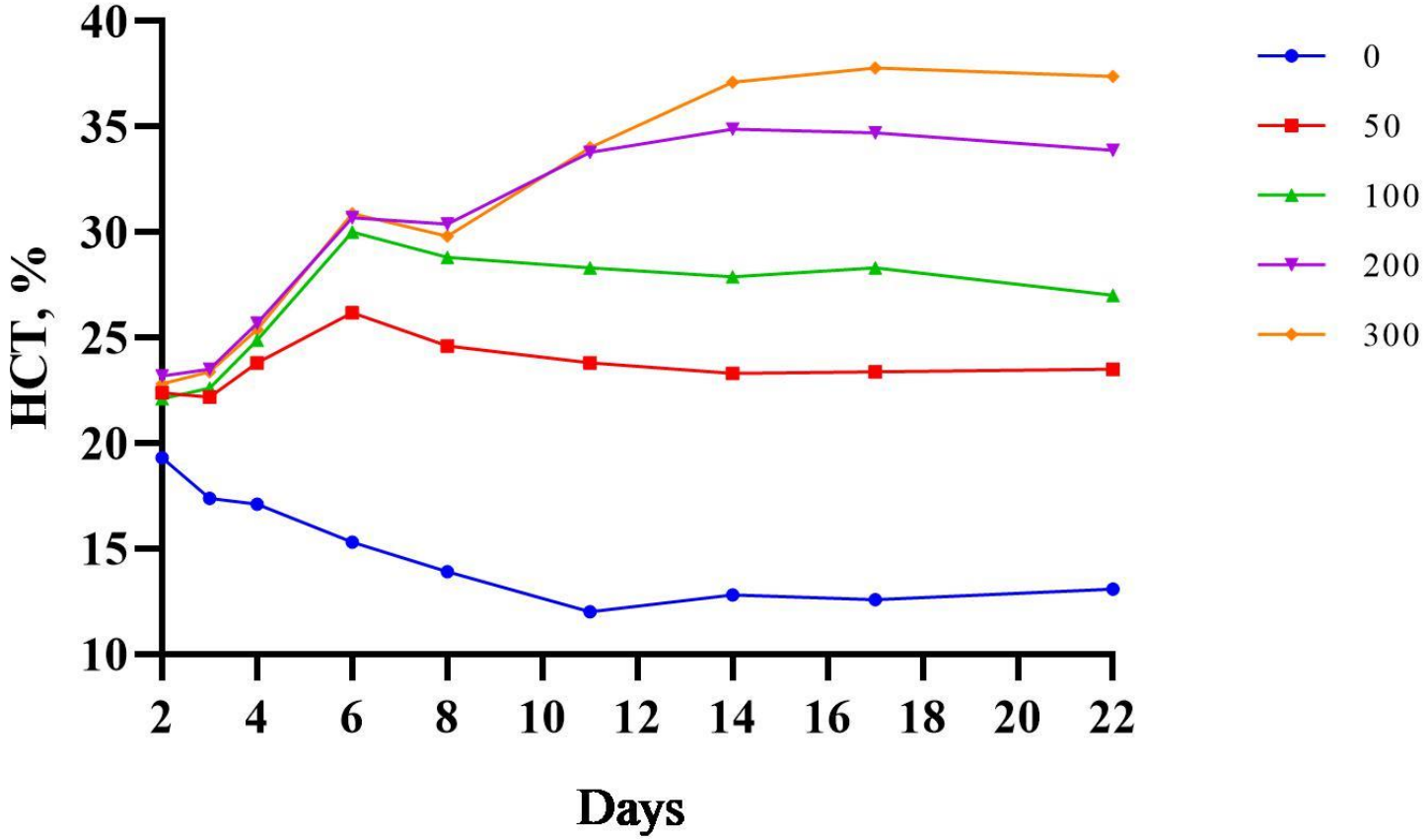

Figure A.3. Effects of iron injection dosage on hematocrit content (HCT) during the preweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P = 0.0002$.

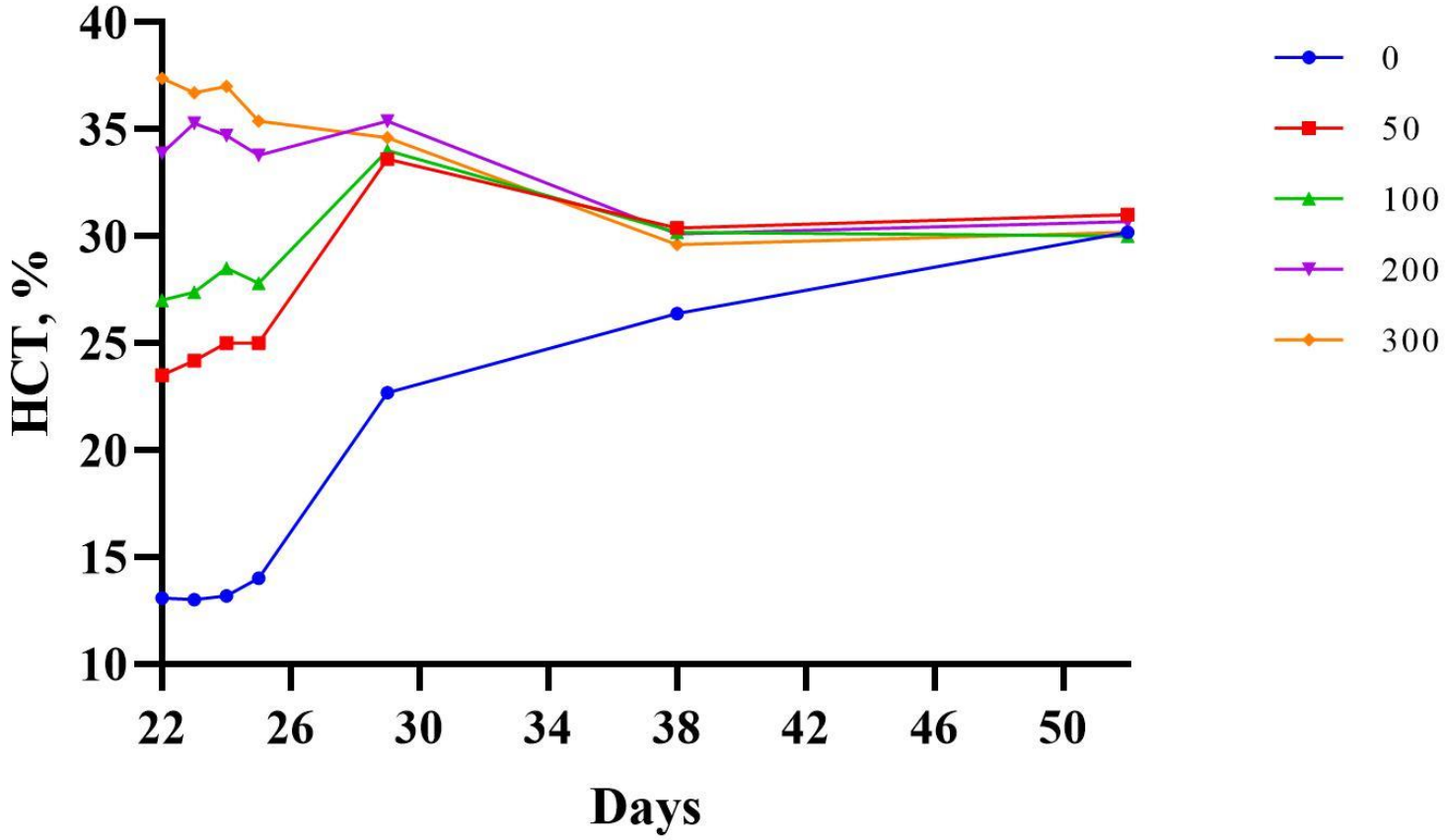

Figure A.4. Effects of iron injection dosage on hematocrit content (HCT) during the postweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$.

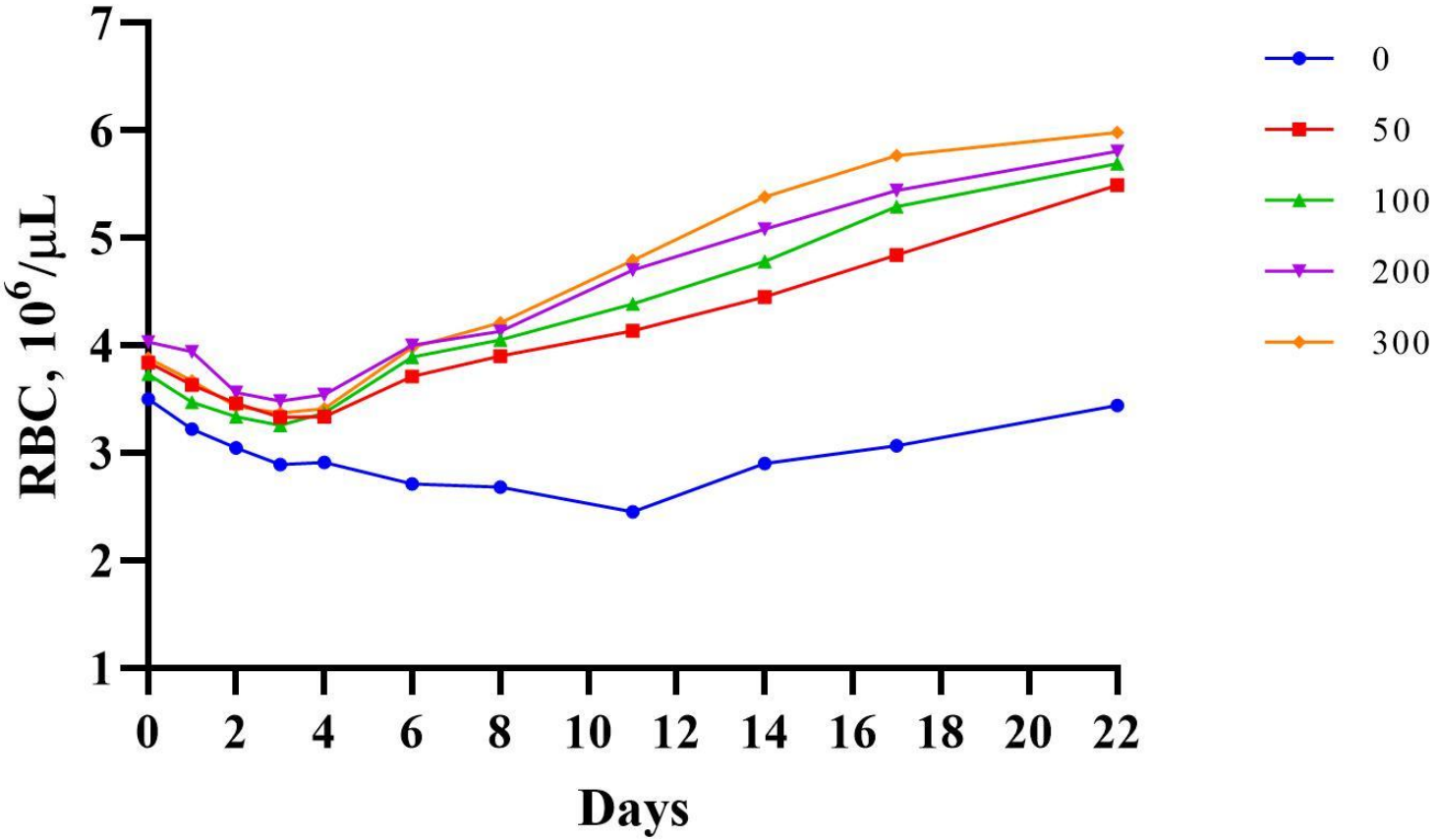

Figure A.5. Effects of iron injection dosage on red blood cell count (RBC) during the preweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$.

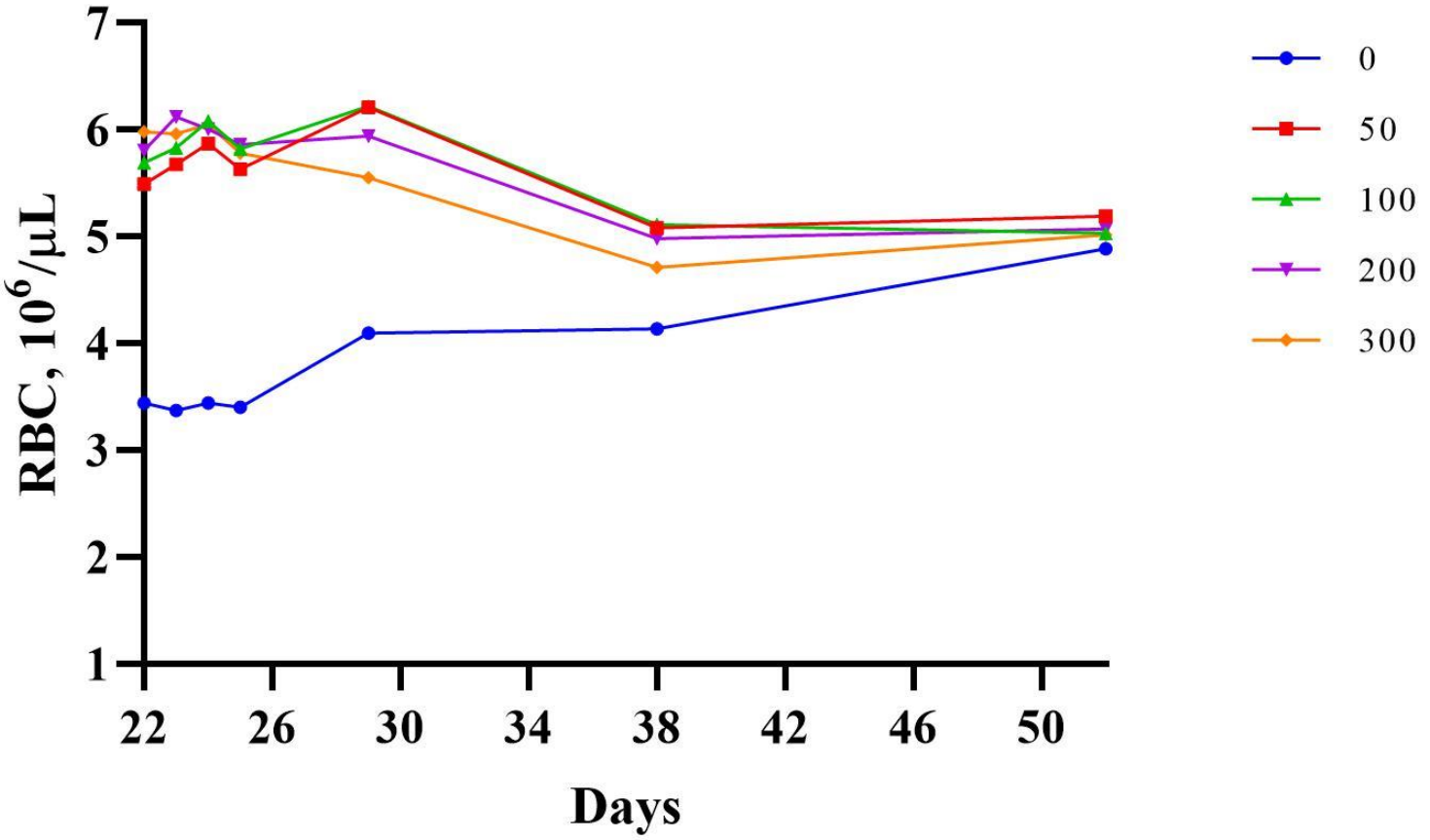

Figure A.6. Effects of iron injection dosage on red blood cell count (RBC) during the postweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P = 0.3117$.

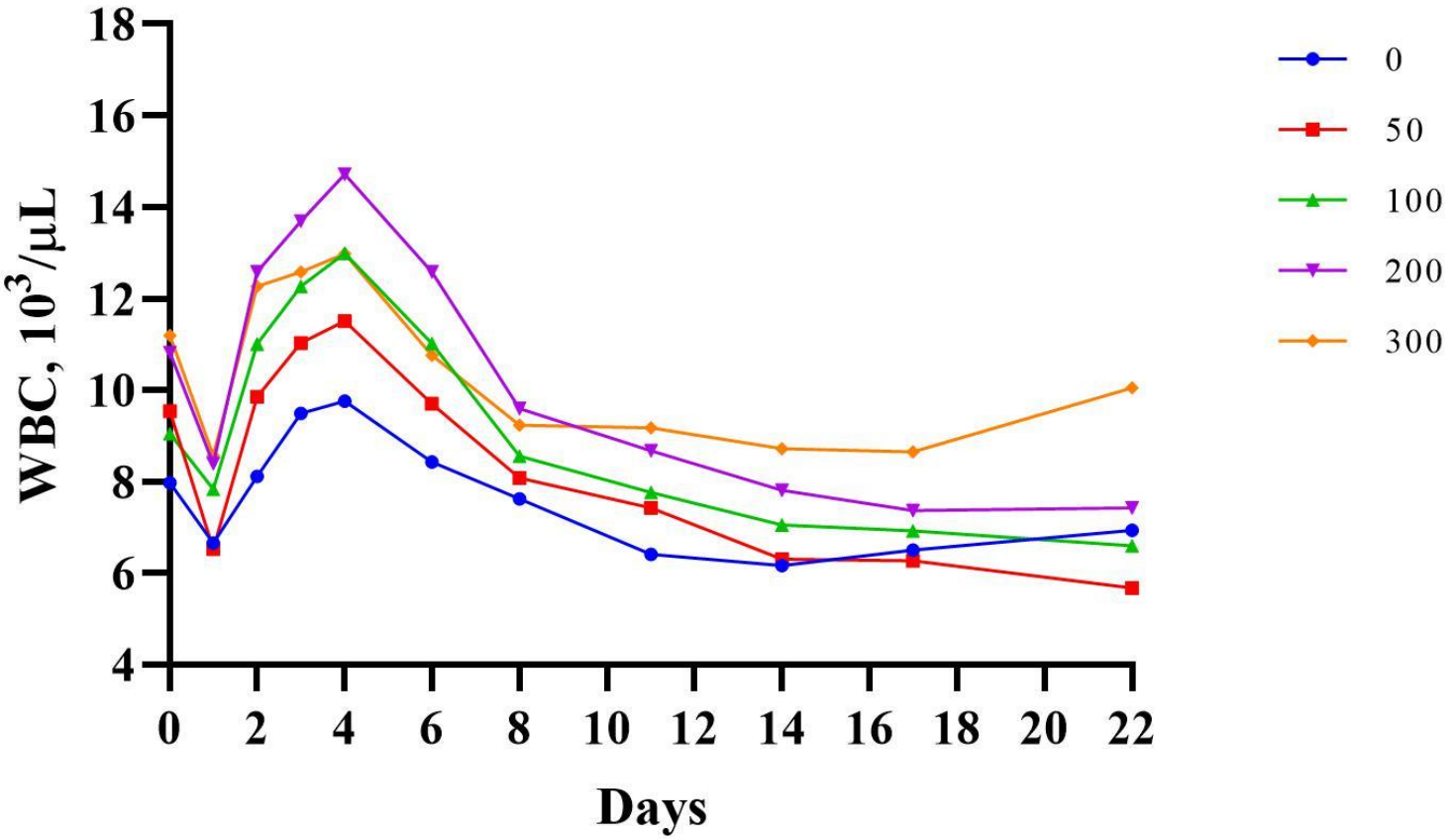

Figure A 7. Effects of iron injection dosage on white blood cell count (WBC) during the preweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P = 0.0012; Day, P < 0.0001; Trt*Day, P = 0.9973; Day contrast: Linear, P < 0.0001 and quadratic P = 0.6683.

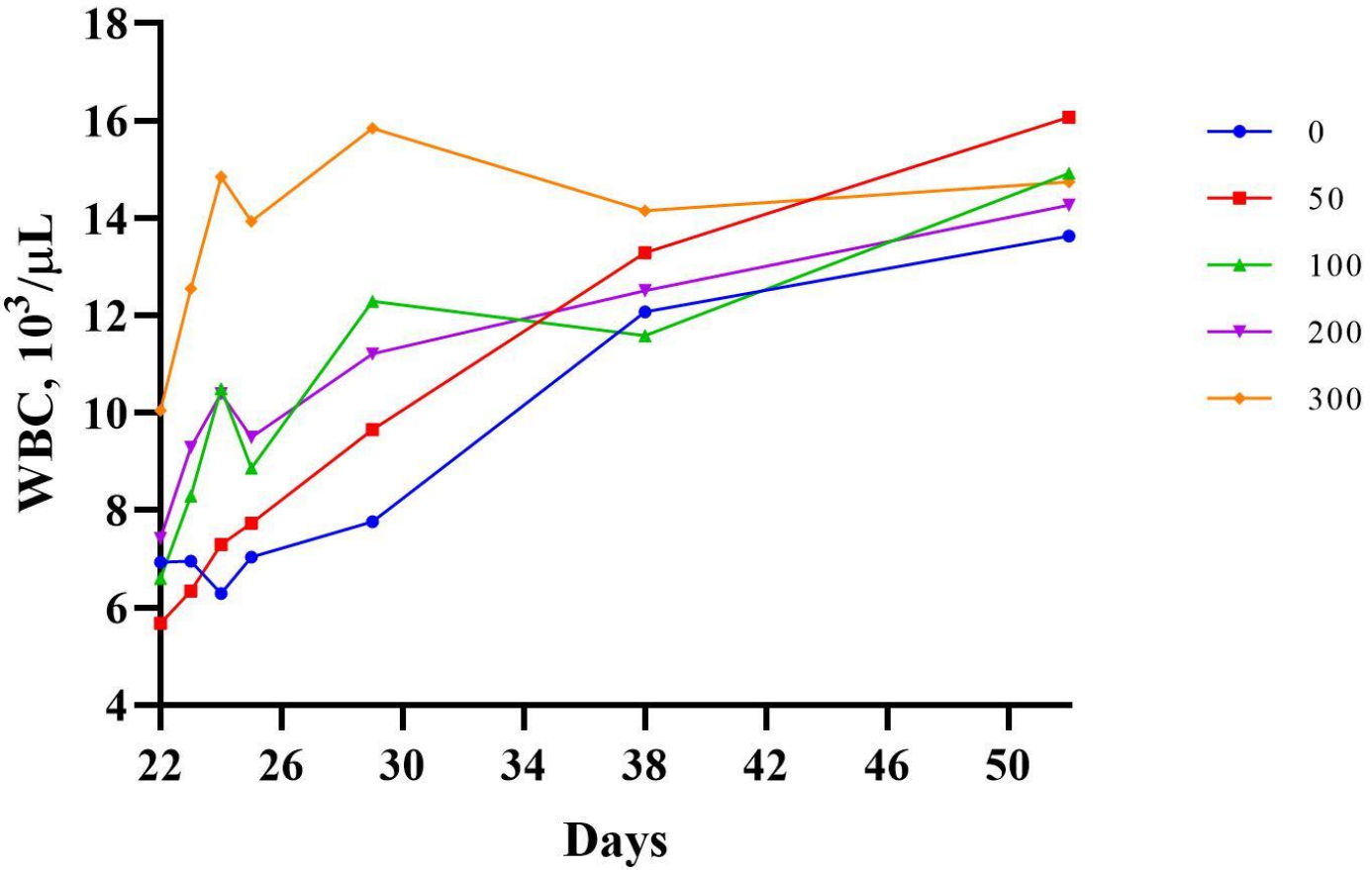

Figure A.8. Effects of iron injection dosage on white blood cell count (WBC) during the postweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P = 0.0073; Day, P < 0.0001; Trt*Day, P = 0.0343; Day contrast: Linear, P < 0.0001 and quadratic P = 0.0031.

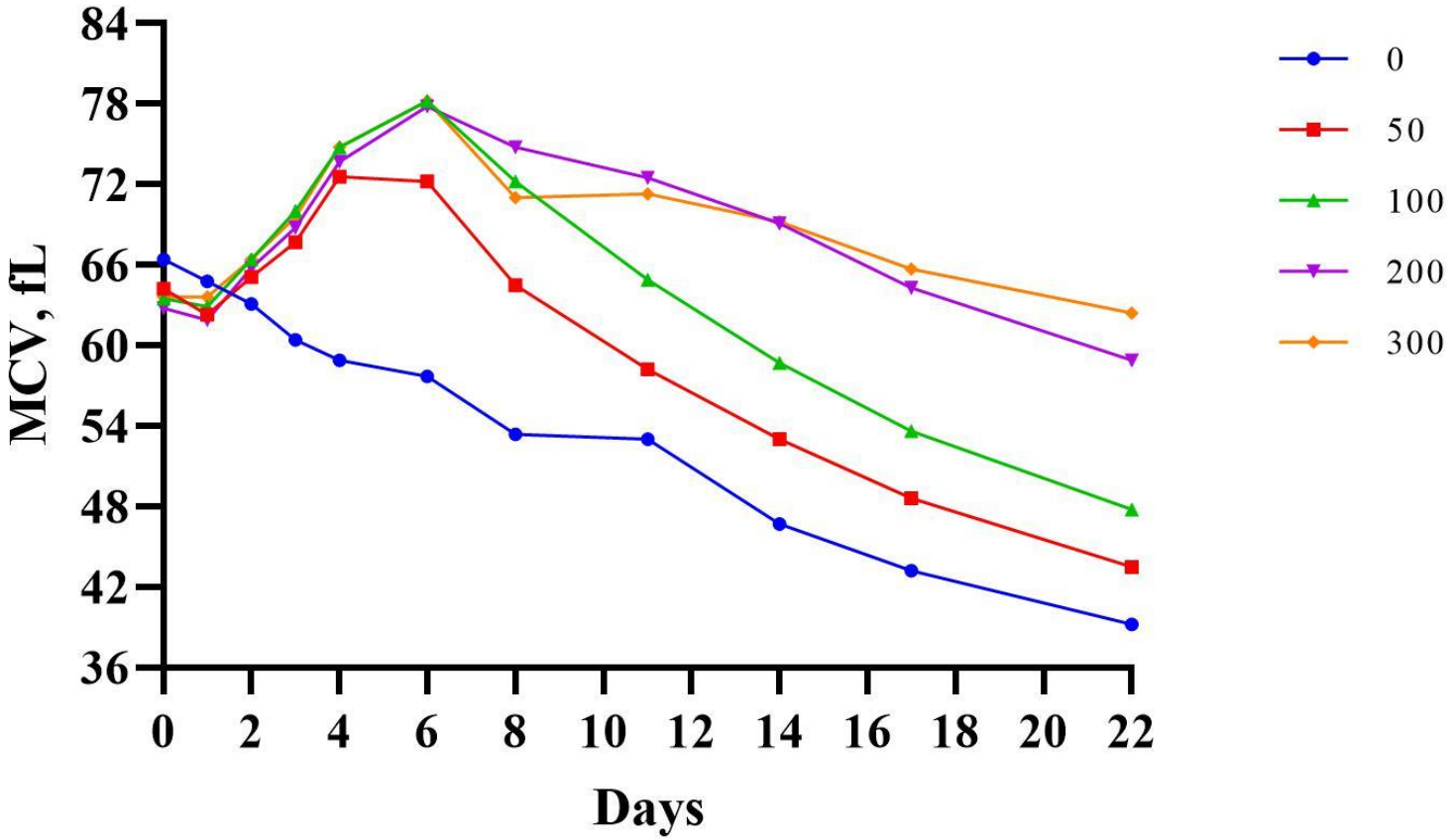

Figure A.9. Effects of iron injection dosage on mean corpuscular volume (MCV) during the preweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$.

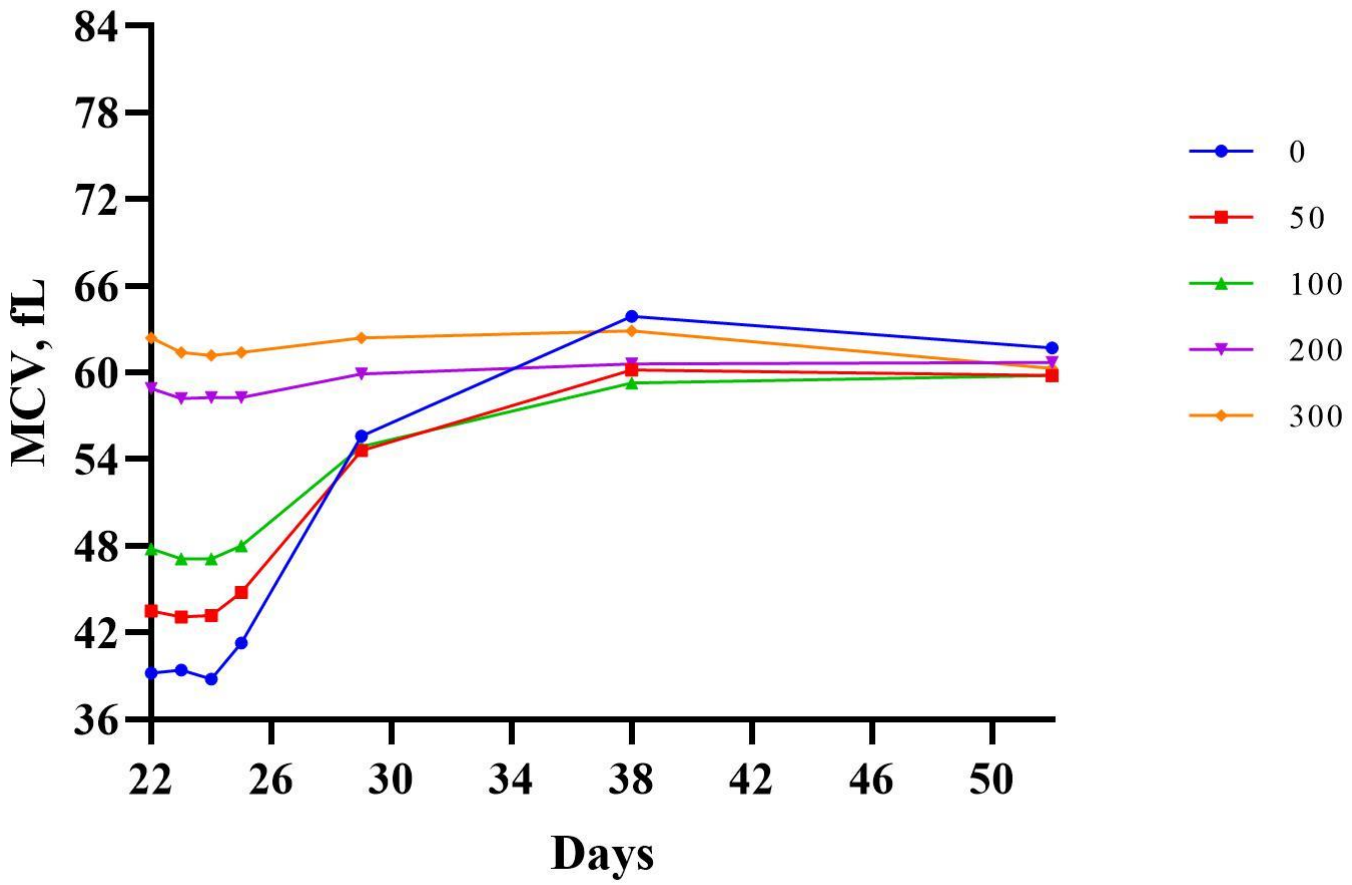

Figure A.10. Effects of iron injection dosage on mean corpuscular volume (MCV) during the postweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$.

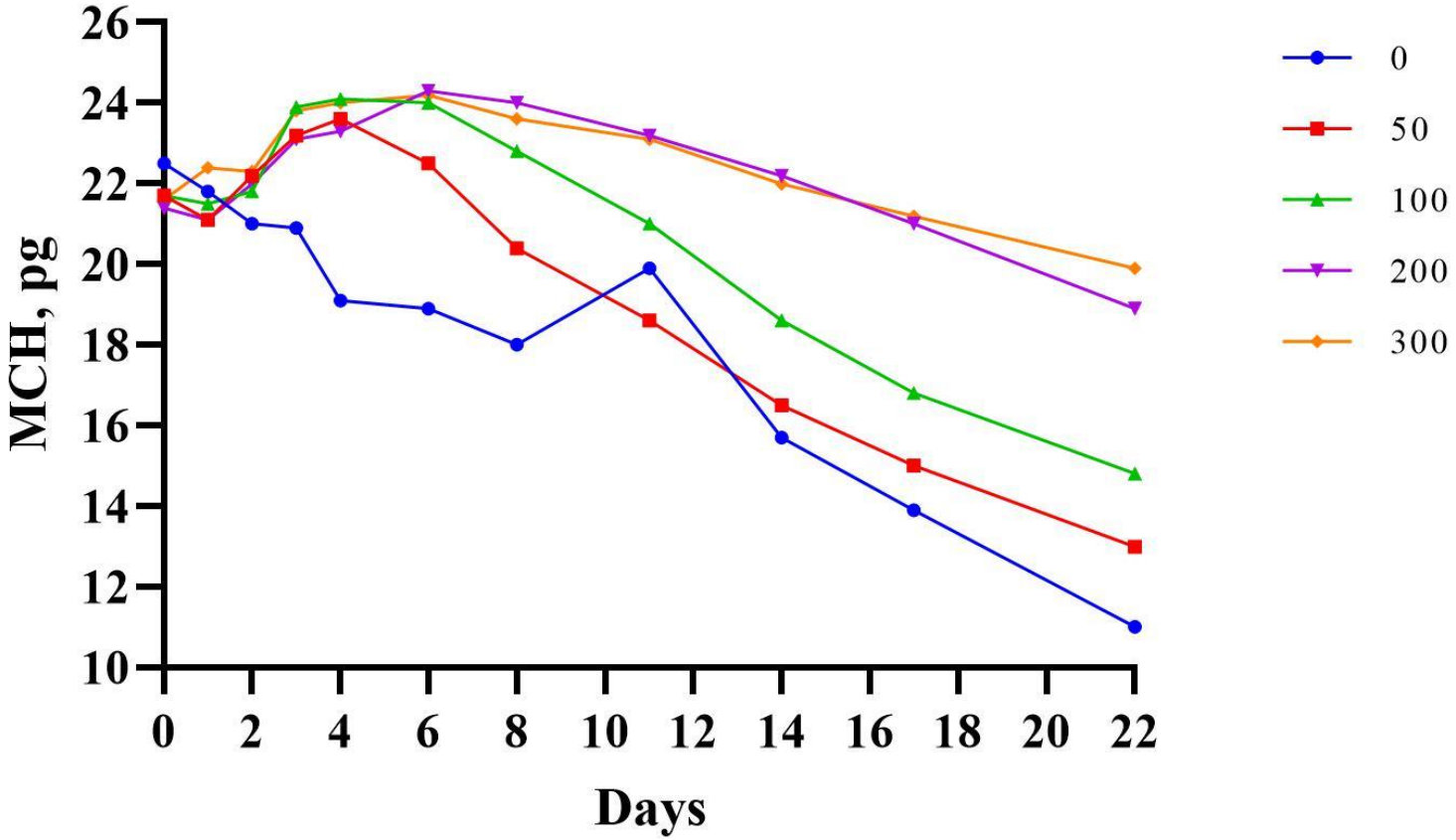

Figure A.11. Effects of iron injection dosage on mean corpuscular hemoglobin (MCH) during the preweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$.

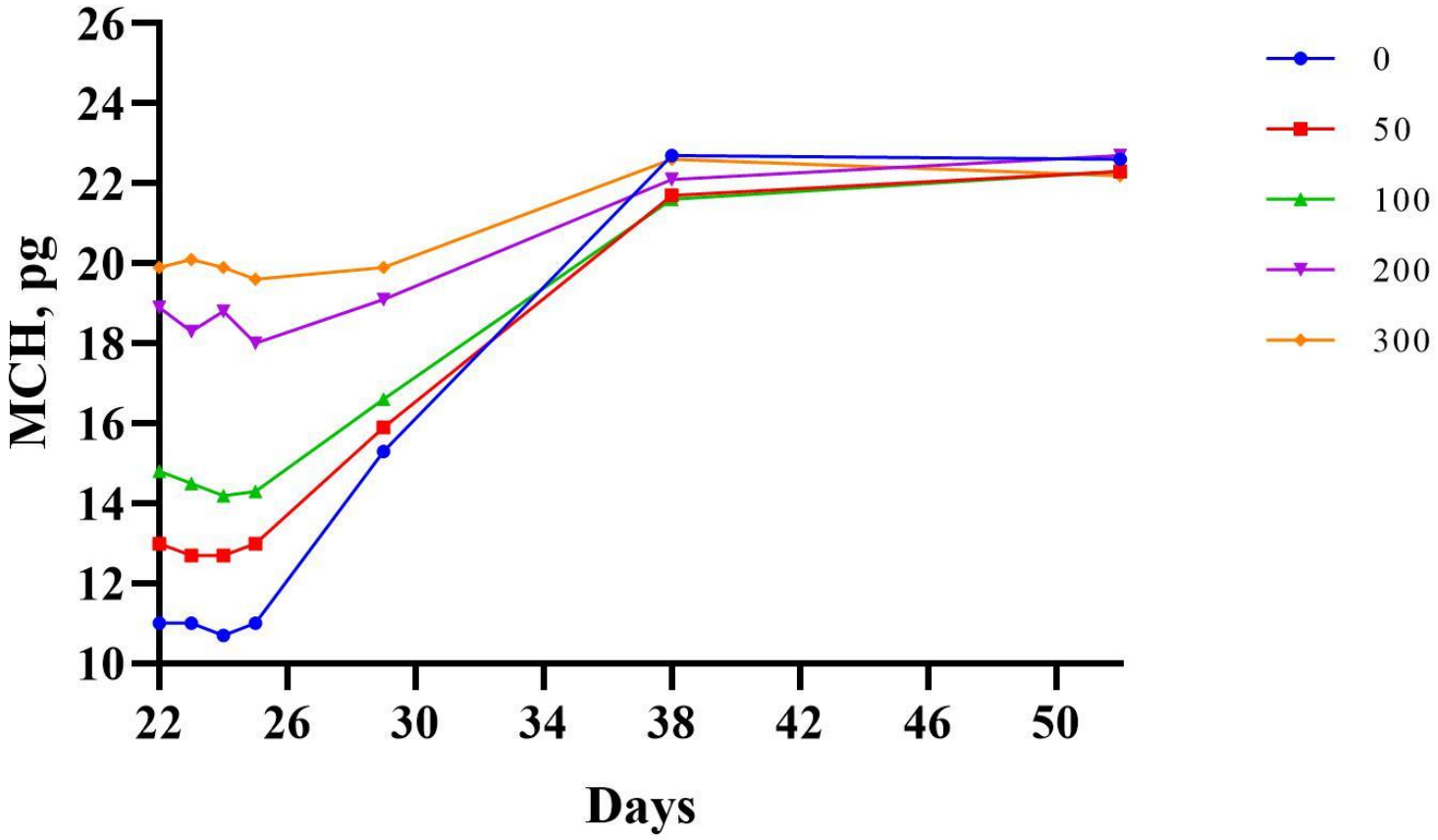

Figure A.12. Effects of iron injection dosage on mean corpuscular hemoglobin (MCH) during the postweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, $P < 0.0001$; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$.

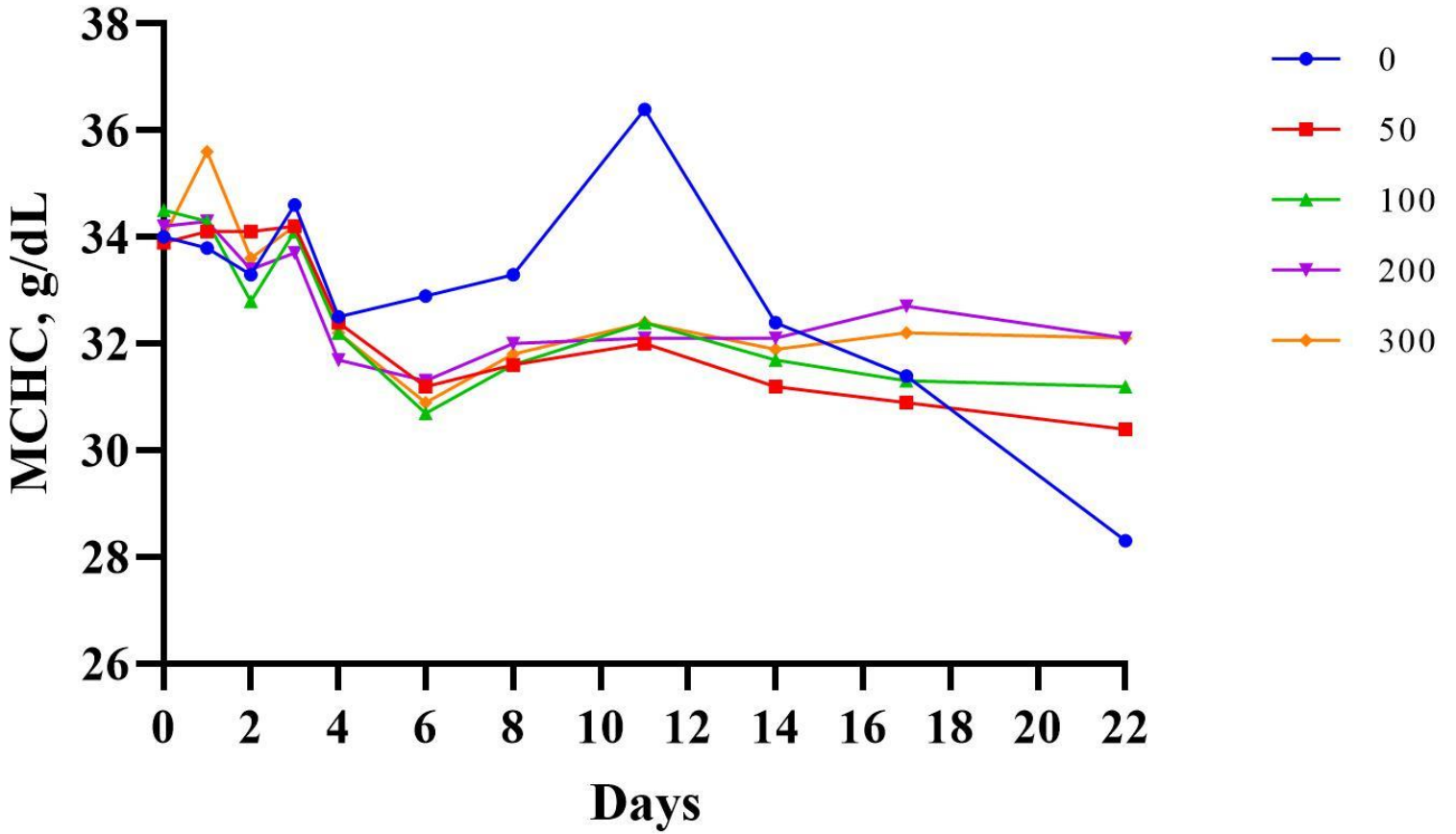

Figure A.13. Effects of iron injection dosage on mean corpuscular hemoglobin concentration (MCHC) during the preweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, P =0.5821; Day, P < 0.0001; Trt*Day, P = 0.0862; Day contrast: Linear, P < 0.0001 and quadratic P = 0.0568.

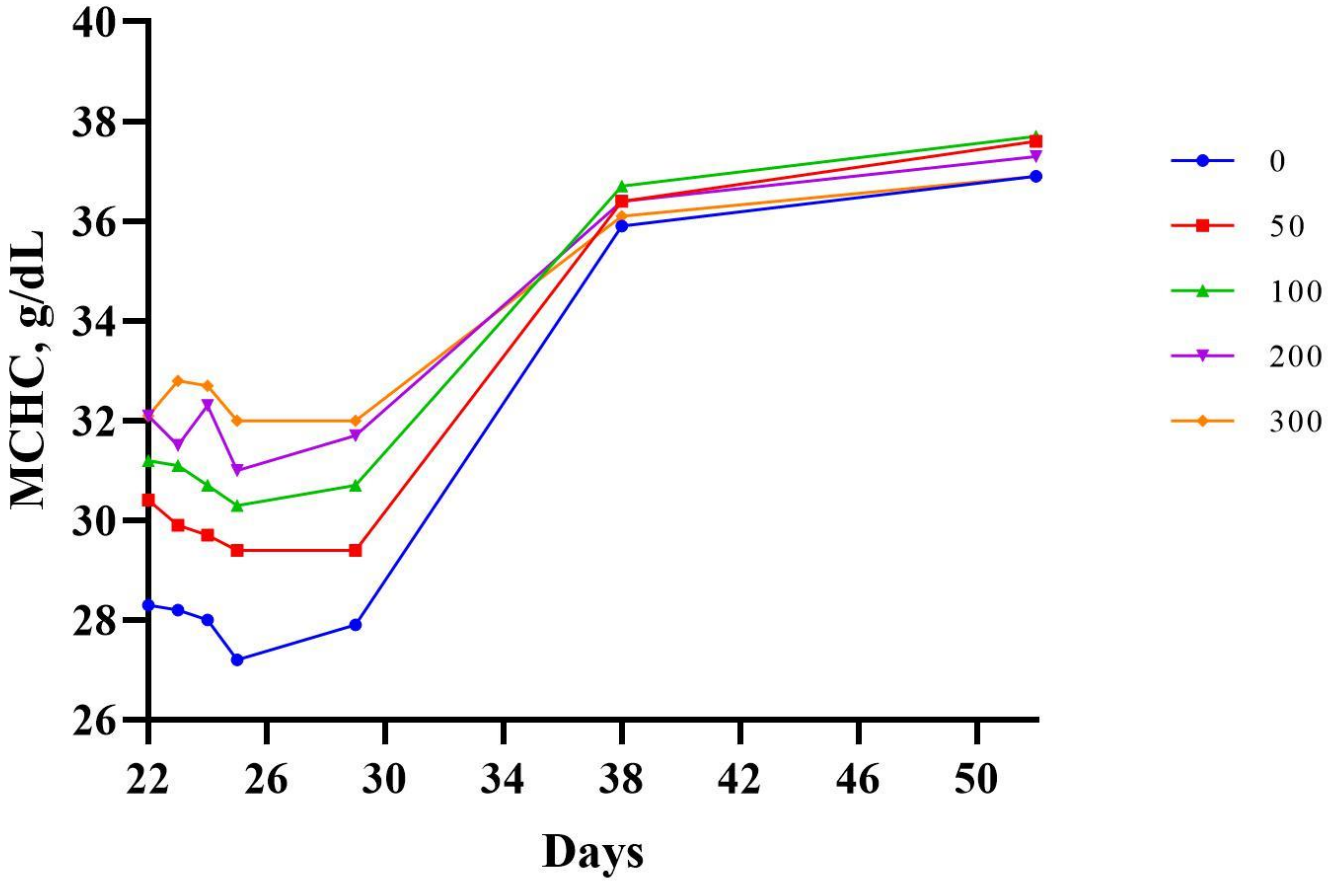

Figure A.14. Effects of iron injection dosage on mean corpuscular hemoglobin concentration (MCHC) during the postweaning period. Iron injection treatments were administered on d 0, pigs were weaned on d 22. P-values: Trt, $P < 0.0001$; Day, $P < 0.0001$; Trt*Day, < 0.0001; Day contrast: Linear, $P < 0.0001$ and quadratic $P < 0.0001$

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Abstracts/Presentations:

ASAS Midwest Section Meeting 2020

788755- Effects of increasing iron dosage at birth on the hematological profile and growth performance of piglets during the lactation period. T.B. Chevalier*, H. J. Monegue, and M. D. Lindemann, University of Kentucky, Lexington, KY

788771- Effects of increasing iron dosage at birth on hematological profile, growth performance, and tissue mineral concentrations of nursery pigs. T.B. Chevalier*, H. J. Monegue, and M. D. Lindemann, University of Kentucky, Lexington, KY

ASAS-CSAS Annual Meeting 2019

660125- Effects of an additional iron injection administered to piglets before weaning. T. B. Chevalier*, H. J. Monegue, and M. D. Lindemann, University of Kentucky, Lexington.

University of Kentucky 9th Annual Poster Symposium

Effects of an additional iron injection administered to piglets before weaning. T. B. Chevalier*, H. J. Monegue, and M. D. Lindemann, University of Kentucky, Lexington.

University of Kentucky 8th Annual Poster Symposium

Assessment of the Iron Status of Young Pigs in a Confinement Herd. T. B. Chevalier*, H. J. Monegue, and M. D. Lindemann, University of Kentucky, Lexington.

ASAS Midwest Section Meeting 2018

514- Assessment of the Iron Status of Young Pigs in a Confinement Herd. T. B. Chevalier*, H. J. Monegue, and M. D. Lindemann, University of Kentucky, Lexington. Journal of Animal Science, Volume 96, Issue suppl_2, 10 April 2018, Pages 274, https://doi.org/10.1093/jas/sky073.511

Awards:

2nd place MS student poster competition

ASAS-CSAS Annual Meeting 2019

1st place MS student poster competition

University of Kentucky 9th annual poster symposium 2019