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EFFECT OF TWO INJECTABLE IRON SOURCES AND THREE ADMINISTRATION PROGRAMS ON GROWTH PERFORMANCE AND HEMOGLOBIN LEVELS IN SUCKLING PIGS

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Abstract

A total of 756 newborn pigs (DNA 600 × 241) from two farrowing groups were used in a 17-d study to evaluate the effects of two different injectable iron sources and three different administration programs on growth performance and hemoglobin levels. A total of 63 litters were utilized with number of pigs per sow equalized on each day of farrowing. One day after birth, all piglets were weighed and divided in half to create light and heavy groups within litter. Lightest and heaviest pigs were removed such that the heaviest six pigs from the light group and the lightest six pigs from the heavy group were allotted in a randomized complete block design to 1 of 6 treatments such that there was 1 light weight block and 1 heavy weight block within litter resulting in 126 replications per treatment with piglet considered the experimental unit. The six treatments consisted of two different sources of injectable iron (iron dextran, Anem-x 100, Aspen Vet, Loveland, CO; gleptoferron, Gleptoforte 200, CEVA Animal Health, Lenexa, KS) administered on d 1 at either 100 or 200 mg per pig or 100 mg administered on d 1 and an additional 100 mg administered on d 11 of lactation. Piglet bodyweight was determined for all the pigs on d 1, 5, 11 and 17 of lactation to calculate ADG. Hemoglobin values were determined for 6 pigs (one for each treatment) per litter on d 5, 11 and 17. The 6 pigs selected within the litter were from the same body weight group and body weight group alternated between sows such that there were a similar number of light and heavy pigs bled for each treatment. Subsequently, 298 pigs from the second farrowing group were weighed and 154 were bled to determine hemoglobin values on day 18 after weaning. During the nursing period, no significant iron source × program interactions ($P > 0.05$) were observed for BW, ADG or hemoglobin values. Piglet BW and ADG were not impacted ($P > 0.05$) by iron source or program during lactation. However, hemoglobin values were greater on d 11 ($P = 0.024$) and 17 ($P = 0.001$) for pigs injected with iron from gleptoferron compared to those injected with iron dextran. Iron injection program also impacted hemoglobin values with d 11 hemoglobin being greater ($P < 0.05$) for those that received 200 mg on d 1 compared to pigs receiving 100 mg. On d 17 of lactation (weaning), hemoglobin was lowest ($P < 0.05$) for pigs that received 100 mg on d 1 compared to those that received 200 mg on d 1 or those that received 100 mg on d 1 and an additional 100 mg injection on d 11. Injectable iron source or program had no impact on hemoglobin or ADG measured 18 d after weaning. In summary, gleptoferron resulted in higher hemoglobin levels and administration of 100 mg iron on d 1 resulted in the lowest hemoglobin levels compared to the administration of 200 mg on d

1 or 100 mg administered on both d 1 and 11. Neither source nor iron injection program impacted piglet growth performance during lactation or postweaning growth or hemoglobin levels.

1. Homeostasis of iron in piglets

Iron is an indispensable element for piglet development, growth and erythropoiesis, which is involved in different biologic processes such as energy metabolism or DNA synthesis (Zhuo et al., 2018). Suckling piglets are characterized by a high iron demand due to their rapid postnatal growth and the rapid expansion of their blood volume (Sperling et al., 2018). Piglets are born with limited iron stores, and sow's milk provide just 1 mg of iron per day, which is insufficient to meet the 7 - 16 mg of daily iron required to maintain adequate growth and prevent anemia (Meng et al., 2025). Consequently, iron supplementation is needed to avoid the depletion of their body iron stores, which results in iron deficiency or clinical anemia within the first days of life (Starzyński et al., 2013). The absorption of dietary iron occurs in the duodenum and in the jejunum via specific transport proteins (Waldvogel-Abramowski et al., 2014). Non-heme ferric iron (Fe3+) is reduced to ferrous iron (Fe2+) by ferrireductases like duodenal cytochrome B (DCYTB) and subsequently it enters in the enterocytes (Vogt et al., 2021; Waldvogel-Abramowski et al.,2014). Once inside the cell, iron can be utilized for metabolic functions, or transferred to the systemic circulation (Vogt et al., 2021). Furthermore, iron, in case of oral supplementation, exhibits an important relationship with the intestinal microbiota, modulating its composition and improving its barrier functions (Ding et al., 2020). In fact, unabsorbed iron impact the microbiota, as it depends on iron for fundamental physiological activities (Dong et al., 2023). The unabsorbed iron can change the microbiota composition developing opportunistic pathogens and decreasing beneficial bacteria such as *Lactobacillus*, compromising the intestinal barrier and leads to an oxidative stress (Ding et al., 2020; Dong et al., 2023). In the body, iron homeostasis is regulated by the duodenal epithelium through modulation of the iron absorption and its recycling in macrophages (Zhuo et al., 2018).

Many proteins play roles in iron metabolism. Transferrin, is a glycoprotein that is the major transporter of ferric iron and maintains iron in its inert state. In fact most of the circulating iron in the blood is bound to transferrin (Vogt et al., 2021). Ferrous iron is oxidized back to ferric iron, enabling the binding to transferrin and subsequent transport through the bloodstream (Vogt et al., 2021; Waldvogel-Abramowski et al., 2014). The transferrin-bound iron is captured by target cells, such as erythrocyte precursors in the bone marrow, through endocytosis thanks to the transferrin receptor 1 (TfR1) (Vogt et al., 2021). The piglets iron homeostasis depends on the recycling of iron from red blood cells, because the daily iron demand for erythropoiesis is higher than the iron absorption from the diet (Starzyński et al., 2013; Waldvogel-Abramowski et al.,

2014). Ferritin also acts as the major iron storage protein in cells such as liver hepatic cells and macrophages (Nairz et al., 2017). This recycling function is performed by macrophages in the spleen and liver, consisting of phagocytose of old erythrocytes followed by extraction of the heme-bound iron, and then either the storage of ferritin or release of it into the plasma via ferroportin (Vogt et al., 2021). Hepcidin is a peptide hormone secreted by the liver that is responsible for the control of systemic iron homeostasis (Fig. 1) (Waldvogel-Abramowski et al., 2014). The hepcidin mechanism of action consists of binding the ferroportin and inducing its degradation, decreasing the absorption of dietary iron by enterocytes (Starzynski et, al. 2013). The systemic iron status regulates the hepcidin synthesis by hepatocytes, increasing in response to high iron loads or inflammation to prevent toxic iron accumulation and decreasing during periods of iron deficiency or accelerated erythropoiesis (Starzyński et al., 2013; Waldvogel-Abramowski et al., 2014). The liver also plays an important role in iron metabolism. Hepatocytes, the most common cell in the liver, have the capacity to synthesize ferritin which represents the major storage localization for absorbed iron (Vogt et al., 2021).

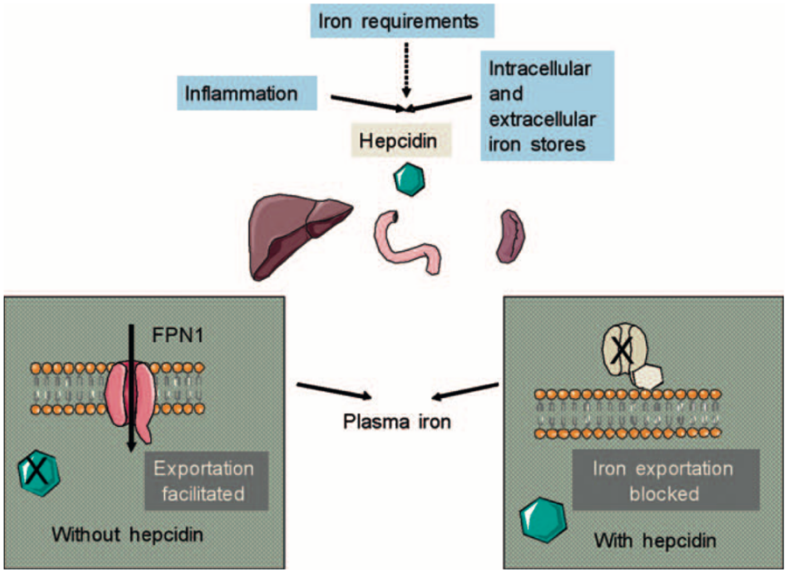


Figure 1. Mechanisms involved in the regulation of hepcidin synthesis (Servier Medical Art: *www.servier.fr/servier-medical-art*)

1.1 Importance of iron for erythropoiesis

Erythropoiesis is the production of oxygen-trans porting red blood cells, and represents the largest portion of the overall body's iron requirement (Vogt et al., 2021). A single erythrocyte contains an elevated number of hemoglobin molecules. Hemoglobin is a protein that transports

oxygen the blood and also acts to release oxygen in tissues for cellular respiration (Sil et al., 2025). Hemoglobin is made up of four polypeptide globin chains and each globin subunit contains a central iron ion that has the capacity to bind oxygen (Farid et al., 2023). Additionally, the inhibition of hepcidin, fundamental for the stimulation of erythropoiesis, is iron dependent (Gou et al., 2020). Thus, iron homeostasis and erythropoiesis are strongly connected. On one side, erythropoiesis increases the absorption of iron through the production of erythroferrone, a hormone that has the capacity to suppress hepcidin expression, on the other, iron is essential to modulate the erythropoietin a glycoprotein hormone produced by the kidneys that stimulates erythropoiesis (Van Vuren et al., 2021; Coffey and Ganz., 2018). Indeed, erythropoiesis as the major body iron utilizer has the capacity to adjust systemic iron homeostasis modulating the expression of erythroferrone. To further understand the synergy between iron and erythropoiesis, it is necessary to understand that iron is the limiting factor for the transition of proerythroblasts into mature erythrocytes within the bone marrow (Muckenthaler et al., 2017). During the differentiation process, erythroid precursors express a high density of Transferrin Receptor 1 (TfR1) to internalize the iron-transferrin complex, a mechanism strictly regulated to ensure that heme synthesis is synchronized with globin chain production (Muckenthaler et al., 2017) (Fig. 2).

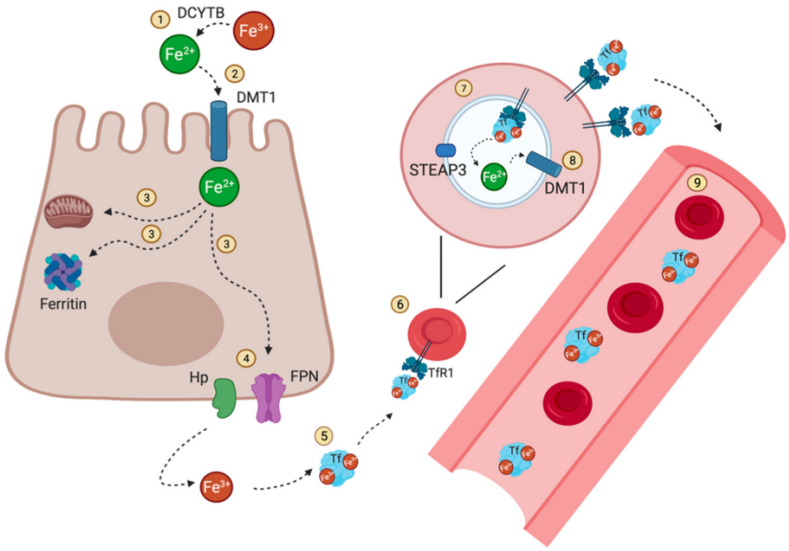


Figure 2. Schematic representation of iron distribution for erythropoiesis, showing the transport of transferrin-bound iron to the bone marrow and its utilization by erythrocyte precursors (Vogt et al., 2021).

If iron availability is insufficient, the heme-regulated eIF2α kinase is activated, inhibiting the globin translation as a protective measure to prevent the accumulation of toxic unpaired globin chains (Chen., 2007). The regulatory feedback loop involving erythroferrone is crucial during periods of erythropoietic function, such as the rapid growth phase in piglets (Kautz et al., 2014). Erythroferrone acts as a suppressor of hepcidin, thus facilitating the iron export from macrophages and intestinal enterocytes into the plasma via ferroportin (Kautz et al., 2014; Arezes et al., 2018). This mechanism mobilizes all the available body stores to meet the demand for iron during neonatal development (Coffey et Ganz., 2018). Therefore, maintaining optimal systemic iron levels is mandatory to support not only the expansion of the red cell mass, but also the overall energetic metabolism of the growing organism (Beard et al., 2001; Muckenthaler et al., 2017).

1.2 Importance of iron for intestinal health and microbiota

Iron is an essential element for the host and the gut microbiota, in fact, iron deficiency leads to gut microbiota imbalances and metabolic disruption (Coe et al., 2021). The composition of intestinal microbiota and consequently intestinal health can be influenced by iron availability due to the iron dependence of microbiota (Paganini et al., 2017). This dependence exists because the majority of bacteria require iron for DNA synthesis and basic metabolic functions (Chen et al., 2020). Consequently, unabsorbed iron can increase the proliferation of opportunistic bacteria, altering the microbial ecosystem in the hindgut (Yilmaz et Li., 2018). In piglets, which are naturally born with low iron stores and characterized by a rapid growth rate, oral or environmental iron is the primary nutrient shaping the microbial structure (Knight et al., 2019). Within the gastrointestinal tract, the host secretes specialized proteins such as lactoferrin to sequester free iron, depriving pathogens of the iron required for colonization (Yilmaz et Li., 2018; Abbas et al.,2022). A study from Dong et al., 2023, demonstrated that iron supplementation can increase or decrease pathogenic and non-pathogenic microorganisms, in particular oral iron can result in an increase of pathogenic bacteria such as *Escherichia coli* and in a decrease in *Lactobacillus*. This shift can increase the risk of post-weaning diarrhea, intestinal inflammation, and compromise barrier function (Chen et al., 2020). However, the relation between gut microbiota and host iron is not fully understood (Dong et al., 2023). Despite this, some studies (Zhang et al., 2023) suggest that the colonic microbiota positively influences host iron absorption mechanisms. In fact, according to those studies, a beneficial microbial fermentation in the hindgut lowers the luminal

pH, preventing the precipitation of ferric iron and improving the solubility and bioavailability of nonheme iron for the host (Zhang et al., 2023).

1.3 Importance of iron for growth performance

There is a strong link between iron and growth performance of piglets, due to different physiological reasons. Piglets are born with very low endogenous iron reserves, and their high postnatal growth rate demands a large amount of iron to support blood volume expansion and myoglobin synthesis in developing muscle tissues (Buyse et al.,2026). Without sufficient iron supplementation, this rapidly increasing demand depletes the body stores, resulting in anemia and lower growth performances (Langley et al., 2025; Buyse et al., 2026). Furthermore, iron is fundamental in the mitochondrial electron transport chain, which generates the ATP required for cellular growth and daily maintenance (Abbas et al.,2022). Some studies (Langley et al., 2025) show that an appropriate iron supplementation can result in higher hemoglobin concentrations at weaning and this may support increased growth performance. Hemoglobin is responsible for oxygen transport to all body tissues, and maintaining an optimal hemoglobin level is necessary to guarantee the metabolic energy neeeded for efficient feed conversion and structural growth (Buyse et al., 2026). Conversely, suboptimal hemoglobin levels reduce oxygen delivery to muscles and organs, which can reduce daily gain and weaning weight and decrease overall vitality (Langley et al., 2025). Moreover, iron can also influence immune cell development, where iron deficiency disrupts gut health and immunity in neonatal pigs which ultimately increases disease susceptibility and consequently has a negative effect on growth performance (Liu et al., 2024). An adequate iron availability is mandatory for the immune system of a sucking piglet to ensure the function, proliferation, and differentiation of macrophages, which are essential to fight pathogens (Abbas et al.,2022). In addition to this, when systemic iron is deficient, the available iron is used preferentially for erythropoiesis over immune defense, which compromises the ability to resist environmental infections (Rouault., 2006). This can result in higher susceptibility to enteric and respiratory diseases, and consequently in lower growth performance before weaning (Buyse et al., 2026; Abbas et al., 2022).

2. Iron deficiency anemia in piglet

There are different types of anemia, all of which iron plays a critical role. Amenia of inflammation is characterized by iron sequestration for erythropoiesis and sideroblastic anemias are characterized by iron accumulation in mitochondria around the nucleus; but above all, iron is fundamental for the most common anemia worldwide, the iron deficiency anemia (IDA) (Camaschella et al., 2022). Iron deficiency anemia (IDA) is a disorder that occurs in piglets during the postnatal period which leads to lower growth performances and increased prewean mortality (Ding et al., 2020). Clinically, iron deficient piglets show wrinkled skin, paleness of the mucous membranes, and are more vulnerable to pneumonia and gastrointestinal infections (Meng et al.,2025). Hemoglobin concentration is used to identify IDA in suckling pigs because IDA is a hypochromic, microcytic anemia responsible for the decreased red blood cell (RBC) indices (Mazgaj et al., 2024). IDA are characterized also by low plasma iron, low transferrin saturation, and high total iron-binding capacity (Sperling et al., 2018). However, blood hemoglobin levels remain the most useful indicator of iron status, as higher levels of hemoglobin support oxygen transport and contribute to heavier weaning weights (Meng et al.,2025). The hemoglobin concentration threshold level for anemia in piglets is commonly defined as 10g/dL (Bardales et al., 2014). Physiological levels of hemoglobin in suckling piglets are variable, and 10 g/dL in blood are considered adequate to prevent growth retardation (Meng et al., 2025). When hemoglobin levels fall between 9 and 11 g/dL, the piglet enters into a borderline deficient condition or first-stage anemia (Sperling et al., 2018). If the hemoglobin concentration is lower than 8 g/dL the piglet enters into subclinical anemia (Meng et al.,2025). Finally, severe or second-stage clinical anemia, which impairs metabolism and retards growth, is diagnosed when hemoglobin concentrations fall to 7 g/dL or less which drastically impairs metabolism and retards growth (Meng et al.,2025; Sperling et al., 2018).

2.1 Physiological origin of IDA

Neonatal iron deficiency anemia (IDA) in pigs represents a problem that affects different breeds and livestock systems (Sperling et al., 2018). The physiological origin of iron deficiency in piglets is the result of different risk factors (Starzyński et al., 2013). Firstly, the sow's milk is a poor source of iron, that contains only 1 mg/L of iron, insufficient to satisfy the piglets requirement which is estimated to be between 7 and 16 mg of iron per day (Mazgaj et al., 2024). Another important factor is the piglets low iron reserves, estimated to be 50 mg in the body; in fact, this hepatic iron

content supports the piglets iron requirement for only the first 3 – 4 days of life (Szudzik e al., 2018; Mazgaj et al., 2024). This low hepatic iron content can be related with genetic selection as modern pigs have been selected for larger litter sizes, high birth weights, and fast postnatal growth, leading to a greater body blood volume that results in a high daily iron demand, moreover, increased litter sizes can result in a lower iron transfer from the pregnant sow to the fetus due to the distribution of iron among a greater number of fetuses (Mazgaj et al., 2024). Finally, the piglet's physiological immaturity of the gastrointestinal tract impairs natural iron uptake (Starzyński et al., 2013). Collectively, all of these factors lead to IDA in newborn piglets.

2.2 IDA after weaning

The weaning period represents one of the most critical and stressful phases in swine production, characterized by the transition from milk to a solid diet (Kim et al.,2017). During this transition, piglets face physiological and nutritional challenges that can lead to iron deficiency anemia (IDA) (Perri et al.,2016). The largest and fastest-growing piglets during the lactation period are the most vulnerable to developing IDA during the weaning (Bhattarai et al.,2015). This occurs because their growth rate dilutes the initial iron reserves (Bhattarai et al.,2015). After weaning, the diet can contain anti-nutritional factors, like phytate, which render the iron chemically unavailable for intestinal absorption (Kim et al.,2017). Furthermore, post-weaning diets are typically supplemented with pharmacological levels of zinc oxide to control enteric infections and post-weaning diarrhea. Some studies (Ding et al., 2020) demonstrated that zinc and iron compete for the same intestinal absorption pathways, so this can reduce the iron bioavailability during the post-weaning period (Perri et al.,2016; Kim et al.,2017).

2.3 IDA impact on Growth

The physiological and economic consequences of IDA are significant in piglets during different productive phases (Meng et al., 2025).

During the lactation period, iron deficiency anemia impairs the animal's development, and a severe iron deficiency negatively impacts structural growth and reduces overall vitality in piglets (Meng et al.,2025; Dong et al.,2020). Suboptimal hemoglobin concentration restricts oxygen delivery to developing muscle tissues, and this compromises growth performance and potentially reduces body weight during the sucking period (Meng et al., 2025). Indeed, one of the most

important consequences of subclinical IDA during early life is a suppressed immune system that increases susceptibility to infectious and parasitic diseases and depresses zootechnical performance (Perri et al.,2016; Leyshon et al.,2019). During the transition to weaning, the piglets are characterized by the highest growth potential under the sow have the highest risk of arriving at weaning with depleted iron reserves and subclinical iron deficiency, reflected in reduced serum iron and increased total iron-binding capacity (TIBC) rather than in overtly anemic hemoglobin values (Bhattarai et al.,2015). In fact, because of their rapid development and of their genetic, these piglets expand their total blood volume at an accelerated rate, which dilutes their iron reserves (Bhattarai et al.,2015). This dilution creates a iron gap at weaning, causing a significant drop in blood hemoglobin concentration during the transition period (Bhattarai et al.,2015). The hematological status of the piglet at weaning is associated with post-weaning growth performance and iron status at weaning may influence nursery performance (Perri et al.,2016). Some empirical evaluations (Perri et al., 2016) reveal that anemics piglets entering the nursery weigh approximately 0.82 kg less after three weeks post-weaning compared to non-anemic piglets. Therefore, avowing subclinical anemia at weaning and maintaining optimal hemoglobin concentrations are fundamental to sustain maximum average daily gain (Meng et al.,2025; Bhattarai et al., 2015).

2.4 Economic loss

The economic impact of IDA represents a challenge for the swine industry (Ding et al.,2020). Iron deficiency suppresses the piglet's immune system and impairs its ability to resist infectious and parasitic diseases, which translates into economic losses for producers (Perri et al., 2016; Leyshon et al., 2019). Specifically, iron deficiency weakens the immune system and makes the anemic animals more susceptible to pneumonia and gastrointestinal infections (Meng et al., 2025). This increased disease susceptibility is associated with higher morbidity and mortality and results in medical and veterinary interventions that represent an additional cost for producers (Perri et al., 2016; Szudzik et al., 2018; Olsen, 2019). Empirical data (Meng et al., 2025) show that piglets receiving no supplemental iron had body weights at day 21of life that were 0.46 to 1.05 kg lower than iron-supplemented piglets (Meng et al., 2025). This loss of weaning weight affects the final value of the pig, as lighter weaners require more time and feed to reach the target slaughter weight (Collins et al., 2017). Producers usually implement iron supplementation strategies to mitigate these economic damages. The main challenge for iron supplementation is to maximize

the efficiency of the mineral while minimizing the costs (Ding et al., 2020). Therefore, finding the most cost-effective and biologically efficient protocol and source is fundamental to preserve farm profitability. (Starzyński et al., 2013; Meng et al.,2025).

3. Prevention of IDA in piglets

The most effective way to combat IDA is prevention, which is more effective than treating the condition after it develops. The goal of anemia prevention is to maintain an optimal physiological iron status and sustain piglet growth (Svoboda et al., 2017). Different strategies have been developed across different stages of swine production to prevent anemia. Maternal supplementation is an approach that is used to increase the transplacental transfer of iron to the fetuses by feeding pregnant and lactating sows with organic iron complexes (Wang et al., 214; McClellan et al., 2025). Another approach is oral administration in suckling piglets. This method is characterized by oral doses of iron salts or chelates to supply the necessary mineral through the gastrointestinal tract (Svoboda et al., 2018; Churio et al., 2018). Finally, the most common and effective preventive method is the parenteral administration of iron-polysaccharide compounds, typically via intramuscular or subcutaneous injections administered during the first days of postnatal life (Svoboda et al.,2017; Lipiński et al., 2026). Independent of the methodology used, the fundamental physiological objective of providing exogenous iron is to supply the rapidly developing animal with adequate systemic iron to support its expansion of blood volume and the intense erythropoietic activity occurring in the bone marrow (Szudzik et al., 2018). Furthermore, any practice balances the supply of iron, ensuring that the iron is utilized for hemoglobin synthesis without leading to oxidative stress (Lipiński et al., 2010).

3.1 Supplementation of iron in pregnant sows

Supplementing pregnant sows with iron is a strategy used to maximize the prenatal accretion of iron in the developing fetuses and to ensure adequate iron stores before birth to prevent neonatal anemia in piglets (McClellan et al.,2025). The efficiency of transplacental iron transport from the sow influences the piglets iron reserves at birth, however, pigs are characterized by a noninvasive, diffuse type of epitheliochorial placenta, meaning that maternal blood is separated from the surface of the chorion and this physiological characteristic renders this process complex (Lipiński et al., 2026). The iron transfer from the sow to the fetus is an indirect process mediated by utoferrin, a bi-iron protein, secreted by the endometrial glandular epithelium. (Lipiński et al., 2026). Because of this biological restriction, iron supplementation in sows, to prevent anemia in piglets are highly debated in different studies (Lipiński et al., 2026).

3.1.1 Oral iron supplementation in sows

The most common maternal strategy of iron supplementation is providing iron through the sow's diet utilizing inorganic iron salts such as ferrous sulfate (McClellan et al., 2025). Modern strategies are characterized by supplementation of organic iron sources such as iron amino acid chelates, polysaccharide-complexed iron and heme iron derived from bovine red blood cells, to avoid the limitations of inorganic salts, such as low bioavailability and their tendency to interact with ingredients in the gastrointestinal tract. (McClellan et al., 2025; Wang et al., 2014). Providing organic iron directly to the sow during gestation can improve maternal iron status, leading to benefits for the litter. For instance, replacing ferrous sulfate with polysaccharide-complexed iron in the sow's diet can increase the sow's serum ferritin levels during gestation and improve maternal iron storage capacity (McClellan et al., 2025). This leads to a more consistent fetal iron supply during gestation, reducing the prevalence of anemia in piglets at birth (McClellan et al., 2025). Despite the effect on the fetal iron transfer, oral iron supplementation of the sow doesn't improve the iron concentration in colostrum or milk (McClellan et al., 2025; Wang et al., 2014). As a result, the oral supplementation of iron in sow diets is not a successful practice to prevent neonatal iron-deficiency anemia (Wang et al., 2014; Peters et al., 2008).

3.1.2 Parenteral iron supplementation in sows

Another approach to maternal supplementation is the parenteral administration of iron in sows, typically utilizing intramuscular injections during gestation (Lipiński et al., 2026). This practice bypasses the gastrointestinal absorption barriers of the sow and elevates systemic iron levels to achieve a higher transfer of iron across the placenta or across mammary glands (Lipiński et al., 2026; Peters et al., 2008). Some empirical studies (Lipiński et al., 2026) show that parental iron administration in pregnant sows can influence the iron status of the piglets (Lipiński et al., 2026). However, even administering elevated doses of iron dextran is not sufficient to counteract the physiological restrictions of the epitheliochorial placenta that limit the transfer of this nutrient to the fetuses (Lipiński et al., 2026). Furthermore, parenteral iron supplementation of the sow during gestation resulted unsuccessfully to increase the iron concentration in the sow's milk (Lipiński et al., 2026).

3.2 Oral iron supplementation in piglets

Oral iron administration represents a non-invasive alternative to parenteral injections for the prevention of iron deficiency anemia in suckling piglets (Svoboda et al., 2018). Oral iron administration can occur by different methods, including direct administration of pastes or liquids into the mouth, voluntary intake in feed, or addition to drinking water (Svoboda et al., 2018). Adding iron to drinking water is not typically deemed successful, as newborn piglets satisfy their fluid requirements through intake of milk (Svoboda et al., 2018). The oral supplementation practice can be based on voluntary intake and offers different advantages such as less discomfort to the animals, less labor associated with individual handling, and elimination of the risk of iatrogenic disease transmission or abscess formation linked to the use of needles (Maes et al., 2011). Whereas individual oral dosing requires handling time and results in behavioral disruptions (Svoboda et al., 2018). Piglets can exhibit rejection behaviors, such as tongue protrusion, head movements, and delayed return to suckling, due to the metallic taste of the iron supplements (Churio et al.,2018). Conversely, voluntary intake methods usually use iron-rich creep feed that can stimulate the natural rooting behavior of the piglets and encourage early consumption (Maes et al., 2011). The immaturity of the neonatal gastrointestinal tract can be a problem for the efficacy of oral supplementation. In fact, during the first week of life, piglets are characterized by a poor expression of non-heme iron receptors and transporters, such as ferroportin (Lipiński et al., 2010; Churio et al.,2018). Various chemical forms of iron have been tested to solve this problem, including iron dextran, iron chelates and bivalent iron salts (Svoboda et al., 2018). However, bivalent iron salts can generate free radicals and cause oxidative stress, causing toxicity (Svoboda et al., 2018). Bioavailable organic sources, such as heme iron derived from bovine erythrocytes, were tested to improve the safety of oral supplementation, since they are absorbed through more efficient mechanisms than non-heme sources (Churio et al., 2018). The most modern solutions include encapsulation technologies, such as spray-drying iron within a maltodextrin matrix that is projected to protect the mineral during gastric transit (Churio et al., 2018). This technique enhances the iron stability and bioavailability and masks its flavor, reducing rejection behaviors and improving the overall iron status of the piglet (Churio et al.,2018). However, despite these technological advancements and welfare advantages, oral iron supplementation is often inconsistent and less successful than parenteral injections in preventing iron deficiency anemia in piglets (Maes et al., 2011). In fact, oral administration is frequently used

as a complementary strategy rather than a replacement for injectable iron protocols (Svoboda et al., 2018).

3.3 Parental iron supplementation in piglets

Injectable iron sources were developed in the 1950s and since that time, parenteral iron administration has become the most common practice used for iron supplementation across the global swine industry (Svoboda et al., 2017; Lipiński et al., 2026). Today, it is universally recognized as the gold standard for the prevention and treatment of neonatal iron deficiency anemia (Szudzik et al., 2018). Parenteral iron administration bypasses the gastrointestinal tract because the parental administration of an exogenous iron source is administered directly to the neonate. This is more effective than oral supplementation which is limited by immature digestive absorption of the piglet (Maes et al., 2011). With parental iron supplementation, every individual piglet receives an exact amount of iron, eliminating the problem of natural feeding behaviors (Maes et al., 2011). This strategy sustains the hematological status of piglets and prevents the economic and health-related losses associated with clinical iron deficiency (Maes et al., 2011; Lipiński et al., 2010). However, despite the fact that parenteral supplementation is consider an effective method, it is a medical intervention and for this reason, the efficiency and safety of this practice depends on different variables such as the chemical source and formulation of the injectable iron, its physiological mode of action, the dosage administered, and the technique of injection (Svoboda et al., 2017). Consequently, while parenteral administration remains the most used practice for anemia prophylaxis, each of these parameters must be evaluated to maximize benefits and avoid complications. Overall, parenteral administration is successful in preventing IDA. This technique guarantees optimal hemoglobin levels and sufficient iron stores to support the physiological demands and rapid growth of modern piglets (Szudzik et al., 2018; Maes et al., 2011).

3.3.1 Iron sources

The chemical formulation of injectable iron sources is one of the most important factors that lead to the selection of the administration protocol to prevent anemia. A fundamental distinction must be made between simple inorganic iron salts and organic macromolecular iron complexes. While inorganic iron salts, such as ferrous sulfate or iron carbonate, are used for oral and dietary supplementation, they are not injected directly into the animal's muscle (Svoboda et al., 2017).

In fact, inorganic ionic iron entering into the systemic circulation can lead to cellular damage and is characterized by high toxicity (Szudzik et al., 2018). For this reason, injectable sources of iron utilize organic complexes, where the iron is bound to a carbohydrate-based stabilizer to create a macromolecular structure that encapsulates the iron (Svoboda et al., 2017). In the swine industry, the two most common injectable organic iron sources are iron dextran and gleptoferron (Svoboda et al., 2017). Iron dextran is the most used injectable iron formulation, and is characterized by a high molecular weight complex composed of a polynuclear iron hydroxide core bound to dextran (Szudzik et al., 2018). Depending on the specific manufacturing process, it can also be formulated using low molecular weight dextran fractions (Lipiński et al., 2010). Gleptoferron represents another organic complex, specifically, a complex of beta-ferric oxyhydroxide and dextran glucoheptonic acid. It has been developed as an alternative to iron dextran, and is characterized by a higher bioavailability and in some studies, showed greater efficacy in correcting hematological profiles and supporting postnatal growth (Svoboda et al., 2017).

3.3.2 Mode of action

High molecular weight iron complexes do not enter the systemic bloodstream directly (Svoboda et al., 2017). The injected compound is transported from the injection site into the lymphatic circulation, here it is taken up by the reticuloendothelial system (Lipiński et al., 2010; Svoboda et al., 2017). Macrophages located in spleen, liver and lymph nodes are the primary cellular actors mediating this uptake (Lipiński et al., 2010; Svoboda et al., 2017). Once inside the macrophages, lysosomal enzymes are responsible for the degradation of the macromolecular complex which releases the active iron core within the cell (Svoboda et al., 2017). After this primary phase, iron can follow two different pathways and can either be sequestered and stored intracellularly in the form of ferritin, or can be exported across the cell membrane into the plasma (Lipiński et al., 2010). Upon entering the circulation, the free iron binds to transferrin and arrives in the bone marrow, where it is utilized for the synthesis of hemoglobin to develop erythrocytes (Svoboda et al., 2017). Iron from an iron dextran source appears in circulating red blood cells within 24 hours of administration and the complex is completely eliminated from the injection site within seven days (Svoboda et al., 2017). Both iron dextran and gleptoferron rely on this exact same macrophage-mediated phagocytosis and lysosomal degradation pathway to become bioavailable (Svoboda et al., 2017).

3.3.3 Doses and administration programs

It is crucial to consider the difference between the volume of the commercial medication administered and the concentration of elemental iron in the product when delivered to the animal. Commercial preparations of iron dextran and gleptoferron can have different concentrations, formulated to contain 100 mg/mL or 200 mg/mL of elemental iron (Chevalier et al., 2021). The dose must be calculated in milligrams of elemental iron, regardless of the liquid volume injected. The most common protocol in swine industry is the administration of a single intramuscular injection of 200 mg of elemental iron, typically between the second and third day of life of the piglet (Lipiński et al., 2026; Szudzik et al.,2018). Administrating the iron 2 or 3 days after birth allows the newborn piglet to consume a sufficient amount of colostrum, which provides antioxidants (Svoboda et al., 2017). This is fundamental to prevent oxidative stress and toxicity that iron can induce (Svoboda et al., 2017). While 200 mg of iron is the standard, different dosages have been tested. Experimental protocols evaluating single doses of 50 mg or 100 mg of injectable iron demonstrated that these amounts are insufficient to sustain optimal hemoglobin concentrations (Chevalier et al., 2021; Svoboda et al., 2017). Conversely, administering high doses, such as 300 mg of iron at birth, saturates hepatic storage but does not significantly improve post-weaning hemoglobin levels and growth performance compared to the 200 mg dose, whereas can increase the risk of toxicity (Chevalier et al., 2021). Because of their rapid growth rate, piglets can deplete even a 200 mg single dose of iron before weaning, and for this reason alternative administration programs characterized by multiple injections have been tested to prevent the late-lactation iron gap (Svoboda et al., 2017). Double-injection programs are characterized by an initial dose, usually on day 3, followed by a booster injection later in the suckling period, such as on day 10 or day 14 (Szudzik et al.,2018). This double-injection program supplements the iron according to the piglet's progressive expansion of blood volume (Lipiński et al., 2010). Furthermore, dividing the total iron into two separate injections prevents the induction of hepcidin synthesis that typically follows a single massive injection (Szudzik et al.,2018). An advantage of the split program is maintaining low hepcidin levels, this ensures that dietary iron absorption mechanisms remain active; however, it does increase the labor needs and piglets need to be picked up a second time to administer the shot (Lipiński et al., 2010; Szudzik et al., 2018).

3.3.4 Limitations of parental iron supplementation

Although the parenteral administration of iron is the most common practice adopted, this practice is associated with some practical disadvantages and can also be associated with some negative physiological effects (Maes et al., 2011). Individually picking up and injecting every piglet is stressful for the animals and requires significant commitment of both labor and time (Maes et al., 2011). This practice also elevates the risk of iatrogenic disease transmission, because of the use of shared needles across multiple animals and improper injection techniques can cause abscesses in the local tissue (Maes et al., 2011). At the physiological level, iron dextran and gleptoferron are safe for healthy piglets, but an overdose or an inability to store the injected iron can lead to acute toxicity (Svoboda et al., 2017). If the transferrin is not able to bind all of the excess iron, the unbound iron catalyzes the production of free radicals and reactive oxygen species, causing oxidative stress that can damage DNA, cellular membranes and proteins (Lipiński et al., 2010; Svoboda et al., 2017). Piglets that are deficient in antioxidants, such as vitamin E and selenium, are more susceptible to iron poisoning (Svoboda et al., 2017; Szudzik et al., 2018). The clinical signs of toxicosis in piglets can include dyspnea, heart failure, muscle tremors, movement incoordination, coma, and even death (Svoboda et al., 2017). Sporadically, the dextran carbohydrate fraction of the complex can induce an anaphylactic shock as an adverse reaction (Szudzik et al., 2018). Furthermore, excessive free iron can suppress the immune system's phagocytic capabilities and promote the growth of bacteria, leading to an increase incidence of infections such as polyarthritis (Svoboda et al., 2017). Massive single doses can lead in an up-regulation of hepcidin, which decreases the absorption of dietary iron (Szudzik et al., 2018). Despite the contraindications and management challenges of parenteral administration, it remains the most effective solution available to the swine industry for improving pre-weaning iron status of suckling pigs (Maes et al., 2011). When executed with correct dosing and hygiene, the physiological benefits of preventing iron deficiency anemia outweigh the associated risks (Svoboda et al., 2017).

4. Assesment of iron status and supplementation efficacy

There are various methods for evaluating the iron status of a piglet, the primary objective is to monitor efficacy of an iron supplementation protocol (Egeli et al., 1998). The iron deficiency develops through three physiological stages: a prelatent stage characterized by a deficit in iron stores, a latent stage characterized by decreased serum iron and transferrin saturation, and a clinical stage manifesting as a drop in circulating hemoglobin concentration (Svoboda et., 2017). To monitor these different stages of deficiency, there are categories of diagnostic indicators, which are divided into biochemical markers, hematological indices and reticulocyte indices (Godyń et al., 2016).

Biochemical markers in the blood serum can be used to detect pre-latent or latent deficiencies before any clinical anemia occurs (Perri et al., 2016). One biochemical marker is serum ferritin that reflects the body's actual iron reserves and can predict iron depletion before physical changes in red blood cells become visible (Churio et al., 2018). Other biochemical indices are transferrin saturation and total iron-binding capacity (TIBC). During iron deficiency, serum iron declines while TIBC rises, resulting in notably low transferrin saturation values (Perri et al., 2016). When it is necessary to diagnose a clinically apparent stage, it is possible to utilize hematological indices, such as hematocrit and the overall red blood cell count (Egeli et al., 1998). However, those indices are not indicators of early iron-deficient erythropoiesis because mature red blood cells have a slow turnover rate of approximately 85 days (Perri et al., 2016). To identify impaired erythropoiesis earlier, it is possible to analyze reticulocyte indices (Godyń et al., 2016). Since reticulocytes only last for about 4 days, parameters like reticulocyte hemoglobin content (CHr) directly reflect recent bone marrow activity (Godyń et al., 2016). This provides an immediate marker of functional iron deficiency before overall hemoglobin levels drop (Godyń et al., 2016). Despite the availability of these indices, hemoglobin concentration remains the most widely used metric for assessing iron status (Perri et al., 2016). The physiological reason is that iron is a fundamental structural component of the hemoglobin molecule, which is required for oxygen transport (Perri et al., 2016). Consequently, a drop in hemoglobin concentration is directly correlated with systemic iron exhaustion (Perri et al., 2016). To evaluate this decline practically, distinct diagnostics are utilized to classify the severity of the deficiency. A hemoglobin concentration above 11 g/dL is indicative of a normal iron status (Perri et al., 2016). When levels fall between 9 g/dL and 11 g/dL, the piglet is considered iron deficient (Perri et al., 2016). Finally, a concentration of 9 g/dL or lower confirms severe clinical anemia (Perri et al., 2016). Tracking

these hemoglobin thresholds is a good solution for herd health. Because using laboratory hematology analyzers is expensive and logistically challenging (Kutter et al., 2012), a practical approach for evaluating the success of iron supplementation at the farms is using Point-Of-Care Testing (POCT) devices (Kutter et al., 2012). Photometric devices, such as the HemoCue system, convert free hemoglobin to azide-methemoglobin and demonstrate excellent accuracy when compared to standard laboratory assessments (Kutter et al., 2012). In contrast, conductivity-based devices, like the i-STAT system, underestimate hemoglobin values in pigs because they are influenced by the hypoproteinemia (Kutter et al., 2012). By utilizing accurate photometric POCT devices, it is possible to screen litters using just a single drop of blood (Kutter et al., 2012). This accessible on-farm practice allows for the immediate validation of iron administration programs, ensuring that the chosen dosage and delivery methods are successfully maintaining optimal physiological hemoglobin levels (Perri et al., 2016).

5. Objective of the thesis

The objective of this study was to evaluate the effects of two different iron sources and three different administration programs on growth performance, and hemoglobin levels in suckling pigs.

6. Materials and methods

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

6.1 Facilities

The Kansas State University Swine Teaching and Research Center is located in Manhattan, Kansas, U.S.A. (Fig. 3). The center operates as a closed-herd, farrow-to-finish research facility, maintaining a commercial-line of approximately 300 sows. The facility is divided into specialized units corresponding to all production phases: breeding and gestation, farrowing, nursery, and growing-finishing. The research infrastructures consist of environmentally controlled rooms equipped with hundreds of specialized pens designed for in vivo clinical trials. This structural configuration allows continuous data collection on performance indicators providing a controlled experimental setting that replicates commercial production conditions.

Specifically, the trial took place in the farrowing area. The farrowing facility consists of an environmentally controlled room equipped with 32 standard farrowing crates. Each crate is designed to minimize pre-weaning mortality and accommodate the lactating sow and her litter (Fig. 4). The crates are fitted with individual sow feeders. Furthermore, the farrowing pens are equipped with thermal mats as supplemental heat sources, to maintain an optimal microclimate for the newborn piglets, to separate watering systems for both the sow and the litter.

Figure 3. Kansas State University Swine Teaching and Research Center

Figure 4. Farrowing crate

6.2 Animals and treatments

A total of 756 newborn pigs (DNA 600 × 241) from two farrowing groups were used in a 17-d study. A total of 63 litters were utilized with number of pigs per sow equalized on each day of farrowing. One day after birth, all piglets were weighed and divided in half to create two body weight groups (light and heavy). The heaviest six pigs from the light group and the lightest six pigs from the heavy group were allotted in a randomized complete block design to 1 of 6 treatments to create a light weight block and a heavy weight block within litter resulting in 126 replications per treatment with piglet considered the experimental unit.

The six treatments consisted of two different sources of injectable iron: iron dextran (Anem-x 100 iron; Aspen Vet, Aspen Vet, Loveland, CO; Fig. 5) and gleptoferrin (Gleptoforte 200, CEVA Animal Health, Lenexa, KS; Fig. 6), administered on day 1 at either 100 or 200 mg per pig or 100 mg administered on day 1 and an additional 100 mg administered on day 11 of lactation. Iron was administered intramuscularly into the cervical musculature of the neck using automatic multi-dose syringes and in accordance with the administration protocol (Fig 7). Creep feed was not offered to suckling pigs and sows were fed a common lactation diet ad libitum.

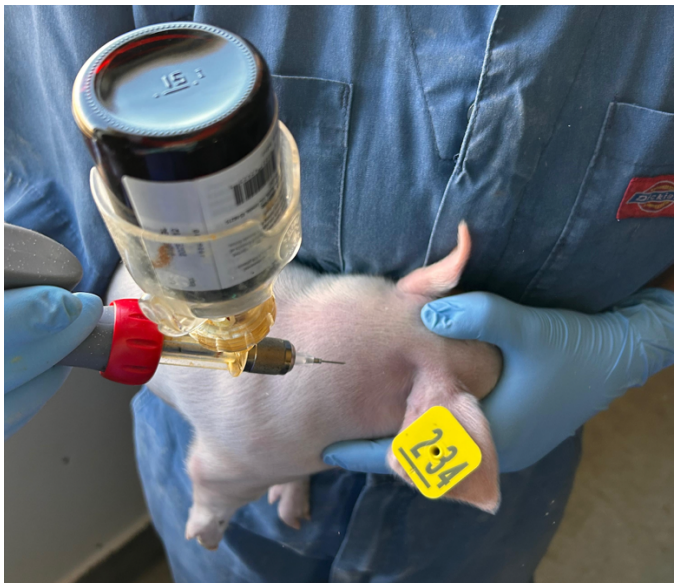


Figure 7. Administration of injectable iron

6.2.1 Identification of piglets

Pigs were identified using the LeeO technology using Radio Frequency Identification Device (RFID). This system use low frequency radio waves (120 and 134.2 KHz) to transmit data between a scanner and the ear tag. The tag utilized was a LF Pig Tag: this tag does not have its own battery but is instead activated by the electromagnetic field generated by an RFID reader, which allows the transmission of the information (Fig 8). The RFID-chipped ear stores the animal number and associated data, such as date of birth, associated sow, genetic line, sex, birth, litter information, cross-fostering events, health treatments and medications. When an animal wearing an ear tag approaches a scanner (Fig.9), a radio signal is generated and transmitted via the scanner's antenna to the tag. The tag then uses this electromagnetic energy to transmit data back to the scanner.

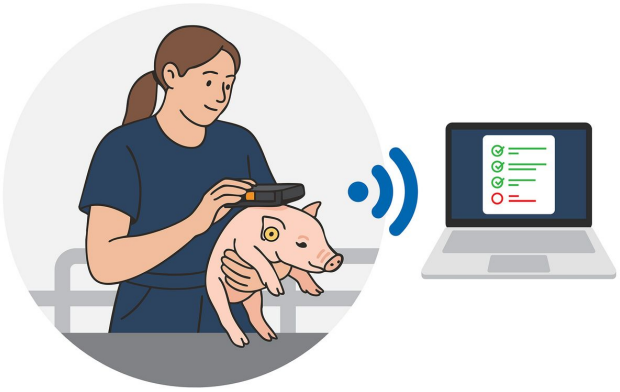


Figure 8. RFID working mechanism (www.nationalhogfarmer.com)

Figure 9. LeeO system LF electronic scanner (www.leeo.eu)

6.3 Farrowing Performance

Individual animal weighing was performed using the weighing equipment integrated with the LeeO identification system. The system combines electronic identification through RFID ear tags with digital weighing devices, that record and automatically associate the weight with the corresponding animal identification number (Fig. 10).

The weight value was then processed by the weighing unit and transferred to the LeeO management system, where it was automatically linked to the individual animal record through the RFID identification number. The collected data were stored in the LeeO database, allowing the creation of an individual digital record for each animal.

Pigs were weighed on d 5, 11 and approximately d 17 (day before weaning) to determine BW change and ADG. There was a 4-d window for farrowing so each litter was weighed according to age, except for the last day where all piglets were weighed on the same day, the day before weaning. Subsequently, 298 pigs from the second farrowing group were weighed 18 d after weaning to determine BW change and ADG post weaning.

Figure 10. Animal weighing and digital weight device.

6.4 Blood samples

Blood samples were collected from the right ear by vein puncture using a sterile needle (Fig. 11) and disposable microcuvette (Fig 12). Samples were analyzed using the HemoCue 201+ Hb analyzer (Fig. 13). Hemoglobin level was determined from 86 randomly selected piglets at d 1 before iron injection and in 6 pigs (one. for each treatment) per litter on d 5, 11 and 17. The 6 pigs selected within the litter were from the same body weight group and body weight group alternated between sows so that there were a similar number of light and heavy pigs bled for each treatment. Additionally, hemoglobin was determined from 154 pigs from the second farrow group 18 d after weaning.

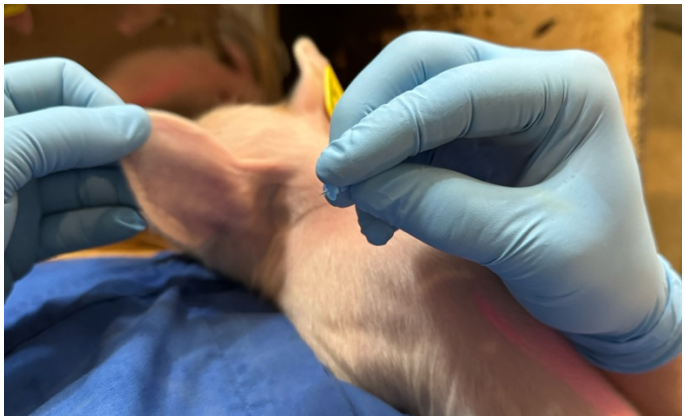


Figure 11. Blood collection

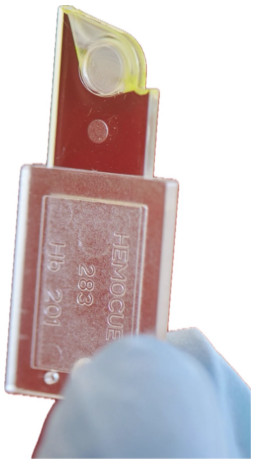


Figure 13. HemoCue 201+ device

Figure. 12 Blood sampling microcuvette

7. Statistical Analysis

Growth data of suckling pigs were analyzed as a randomized complete block design in a 2 × 3 factorial arrangement, using the individual pig as the experimental unit, iron source and iron program as fixed effects, and farrowing group and sow as random effects using SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Growth data of nursery pigs were analyzed separately using the same procedures on the subset of 298 piglets that were followed into the nursery. Hemoglobin values from suckling pigs were measured as a repeated measure within pig with the individual pig as the experimental unit, treatment and timepoint as fixed effects and group, sow, and pig as random effects. Hemoglobin values of nursery pigs were analyzed separately using the same procedures on a smaller subset of 154 piglets that were followed into the nursery and had previously been bled for hemoglobin determination. Treatment differences were considered significant at $P \leq 0.05$.

8. Results

Throughout the trial, there were no iron source × injection program interactions observed ($P > 0.05$) for any response criteria (Table 1). Neither iron source nor injection program influenced ($P > 0.05$; Table 2) suckling piglet BW or ADG. Hemoglobin values were greater on d 11 ($P = 0.024$) and 17 ($P = 0.001$) for pigs injected with iron from gleptoferron compared to those injected with iron dextran. Iron injection program also impacted hemoglobin values with d 11 hemoglobin being greater ($P = 0.001$) for those that received 200 mg on d 1 compared to pigs receiving 100 mg. On d 17 of lactation, hemoglobin was lowest for pigs that received 100 mg on d 1 compared to those that received 200 mg on d 1 or those that received 100 mg on d 1 with an additional 100 mg injection on d 11 with the latter two treatments not differing. Injectable iron source or program had no impact on hemoglobin or ADG measured 18 d after weaning. In summary, neither source nor iron injection program impacted piglet growth performance during lactation, but pigs receiving iron from gleptoferron had increased hemoglobin levels at weaning. Providing at least 200 mg of iron either at d 1 or splitting the dose by providing 100 mg at d 1 and d 11 resulted in higher weaning hemoglobin compared to only providing 100 mg on d 1. For those pigs that were followed into the nursery, iron injection source and program had no impact on postweaning growth or hemoglobin levels.

Table 1. Interactive effects of iron sources and injection program on growth performance and hemoglobin levels in suckling pigs[1]

	Iron source									
	Iron dextran, mg/pig			Gleptoferron, mg/pig						
d 1	100	200	100	100	200	100		*P* =		
d 11	--	--	100	--	--	100	SEM	Program × source	Program	Source
BW, lb										
d 1	3.54	3.53	3.54	3.54	3.54	3.54	0.08	0.997	0.998	0.977
d 5	5.15	5.18	5.08	5.11	5.13	5.17	0.11	0.583	0.912	0.983
d 11	8.66	8.71	8.47	8.72	8.58	8.79	0.17	0.226	0.892	0.450
d 17	12.79	12.70	12.60	12.76	12.60	12.88	0.26	0.590	0.802	0.733
ADG, lb										
d 1 to 5	0.36	0.37	0.34	0.35	0.36	0.36	0.032	0.330	0.815	0.938
d 5 to 11	0.64	0.64	0.61	0.66	0.63	0.66	0.034	0.087	0.538	0.174
d 11 to 17	0.77	0.76	0.76	0.77	0.76	0.78	0.076	0.946	0.835	0.697
d 0 to 17	0.57	0.56	0.55	0.57	0.55	0.57	0.052	0.444	0.775	0.605
Hemoglobin, mg/L [2]										
d 1[3]				10.11 ± 1.78						
d 5[4]	8.6	8.8	8.6	8.5	8.8	8.6	0.151	0.953	0.178	0.930
d 11[4]	9.5	10.0	9.6	9.8	10.2	9.8	0.134	0.765	0.001	0.024
d 17[4]	9.4	11.0	11.3	10.2	11.4	11.4	0.166	0.103	< 0.001	0.001

[1] A total of 756 piglets (DNA 241 × 600) were used in a 17-d study to evaluate the impact of two iron sources and three iron injection programs on growth performance and hemoglobin levels. Piglet was the experimental unit and there were 126 replications per treatment.

[2] Blood samples were collected from the ear vein of the same piglet within each treatment and crate on the indicated days. Hemoglobin concentrations were determined using the HemoCue 201+ system (HemoCue AB, Ängelholm, Sweden).

[3] Before the first iron injection was administered, hemoglobin concentrations were analyzed in 86 randomly selected piglets. Values represent the mean ± standard deviation.

[4] Program × Source × Timepoint, $P = 0.502$; Program × Timepoint, $P < 0.001$; Source × Timepoint, $P = 0.031$; Program × Source, $P = 0.380$; Program, $P < 0.001$; Source, $P = 0.009$; Timepoint, $P < 0.001$.

Table 2. Main effect of iron sources and injection program on growth performance in suckling pigs[1]

	Iron source				Iron program (dose, mg/pig)				
					d 1	100	200	100	
	Iron dextran	Gleptoferron	SEM	*P* =	d 11	---	---	100	SEM	*P* =
BW, lb										
d 1	3.5	3.5	0.07	0.977		3.5	3.5	3.5	0.07	0.998
d 5	5.1	5.1	0.09	0.983		5.1	5.2	5.1	0.10	0.912
d 11	8.6	8.7	0.13	0.450		8.7	8.6	8.6	0.14	0.892
d 17	12.7	12.7	0.21	0.733		12.8	12.6	12.7	0.22	0.802
ADG, lb										
d 1 to 5	0.36	0.36	0.031	0.938		0.36	0.36	0.35	0.031	0.815
d 5 to 11	0.63	0.65	0.032	0.174		0.65	0.63	0.64	0.032	0.538
d 11 to 17	0.76	0.77	0.075	0.697		0.77	0.76	0.77	0.075	0.835
d 0 to 17	0.56	0.56	0.051	0.605		0.57	0.56	0.56	0.051	0.775
Hemoglobin, mg/L[2]										
d 5	8.7	8.7	0.151	0.930		8.5	8.8	8.6	0.151	0.178
d 11	9.7	9.9	0.134	0.024		9.6[b]	10.1[a]	9.7[b]	0.134	0.001
d 17	10.6	11.0	0.166	0.001		9.8[b]	11.2[a]	11.4[a]	0.166	<0.001

[1] A total of 756 piglets (DNA 241 × 600) were used in a 17-d study to evaluate the impact of two iron sources and three iron injection programs on growth performance and hemoglobin levels. Piglet was the experimental unit and there were 63 replications per iron source treatment and 42 replications per iron injection program treatment.

[2] Blood samples were collected from the ear vein of the same piglet within each treatment and crate on the indicated days. Hemoglobin concentrations were determined using the HemoCue 201+ system (HemoCue AB, Ängelholm, Sweden).

Table 3. Effect of iron sources and administration programs on growth performance and hemoglobin levels of pigs pre-and post-weaning[1]

	Iron source									
	Iron dextran, mg/pig			Gleptoferron, mg/pig						
d 1	100	200	100	100	200	100		*P* =		
d 11	--	--	100	--	--	100	SEM	Program × source	Program	Source
BW, lb										
d 1	3.53	3.56	3.54	3.53	3.57	3.58	0.131	0.979	0.893	0.796
d 5	5.14	5.18	5.19	5.18	5.24	5.24	0.179	0.995	0.897	0.670
d 11	8.56	8.58	8.44	8.69	8.63	8.69	0.265	0.896	0.959	0.391
d 17	12.63	12.79	12.42	12.93	12.85	12.87	0.389	0.809	0.841	0.297
d 35	20.74	21.42	21.15	21.19	21.42	20.88	0.665	0.737	0.558	0.871
ADG, lb/d										
d 1 to 5	0.33	0.32	0.33	0.33	0.33	0.33	0.019	0.953	0.924	0.655
d 5 to 11	0.69	0.68	0.65	0.70	0.68	0.69	0.025	0.573	0.510	0.253
d 11 to 17	0.83	0.87	0.82	0.87	0.86	0.86	0.036	0.590	0.581	0.231
d 0 to 17	0.61	0.62	0.60	0.63	0.62	0.63	0.022	0.714	0.792	0.255
d 17 to 35	0.45	0.48	0.49	0.46	0.48	0.45	0.025	0.326	0.454	0.433
Hemoglobin, mg/L[2]										
d 5[3]	8.76	8.76	8.51	8.60	8.72	8.60	0.271	0.884	0.754	0.886
d 11[3]	9.30	9.87	9.73	9.54	9.96	9.95	0.231	0.918	0.054	0.298
d 17[3]	9.38	10.76	11.36	10.17	11.58	11.08	0.268	0.042	< 0.001	0.030
d 35[3]	10.20	10.13	10.37	10.33	10.68	10.32	0.288	0.517	0.884	0.344

[1] A subset of pigs from the larger experiment were followed through the early nursery stage to evaluate growth performance from weaning to d 35 of age and d 35 hemoglobin concentration. For this, a total of 298 piglets (DNA 241 × 600) were used with the main source effect represented by 142 and 156 replications for dextran and gleptoferron, repectivly. For program, there were 95, 104, and 99 replications for the 100 mg, 200 mg, and 100 + 100 mg programs, respectively.

[2] Blood samples were collected from the ear vein of the same piglet within each treatment and crate on the indicated days. Hemoglobin concentrations were determined using the HemoCue 201+ system (HemoCue AB, Ängelholm, Sweden). Hemoglobin was analyzed on a subset of the population that was followed into the nursery, with 154 piglets selected due to having previously collected blood for hemoglobin analysis preweaning. For source, there were 73 and 81 replications for dextran and gleptoferron, repectivly.

For program, there were 49, 55, and 50 replications for the 100 mg, 200 mg, and 100 + 100 mg programs, respectively.

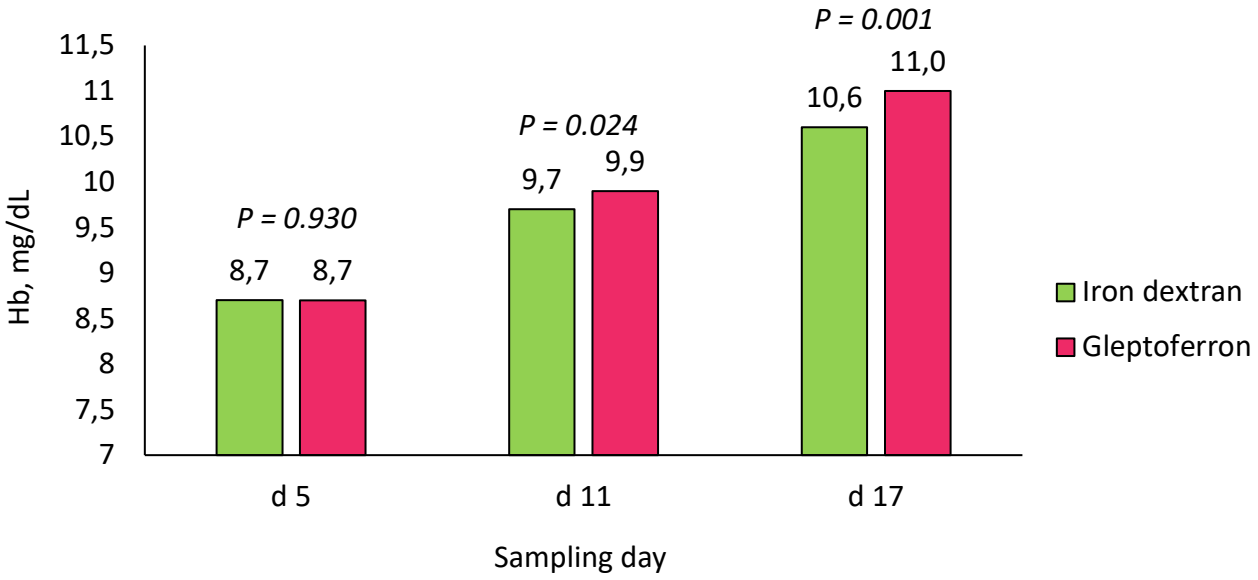
[3]Program × Source × Timepoint, $P = 0.399$; Program × Timepoint, $P < 0.001$; Source × Timepoint, $P = 0.447$; Program × Source, $P = 0.399$; Program, $P < 0.001$; Source, $P = 0.076$; Timepoint, $P < 0.001$.

9. Discussion

Piglets injected with gleptoferron resulted in higher hemoglobin levels (9.9 g/dL) compared with piglets injected with iron dextran (9.7 g/dL) at d 11 and at weaning (11.0 vs 10.6 g/dL).

Whereas iron source did not affect body weight or average daily gain, and no iron source × program interactions were found for any response criterion.

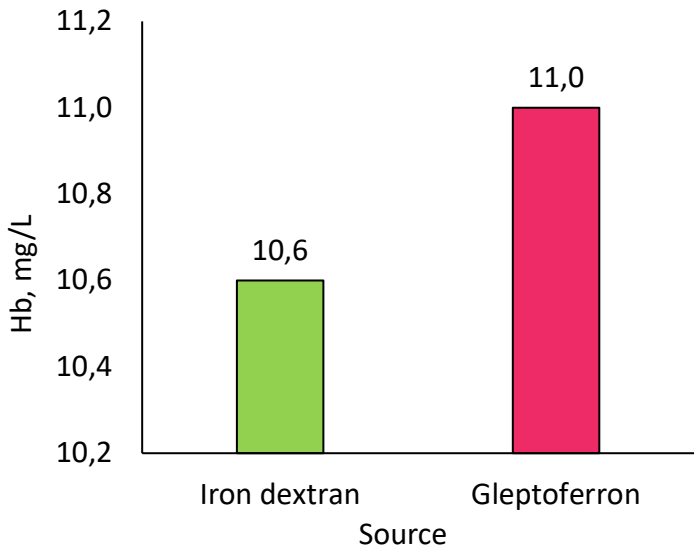
The higher hemoglobin levels obtained with gleptoferron (Graph 1) agree with other studies (Sperling et al., 2018), in which a single 200 mg injection of gleptoferron resulted in higher hemoglobin levels (11.30 g/dL) than 200 mg of iron dextran (10.05 g/dL). The reason for those results is the greater bioavailability of gleptoferron, that achieves a 4.6 times higher total iron absorption than iron dextran at the same dose of 200 mg (Morales et al., 2018). However, those results are no supported by Perri et al., 2016 since in this commercial trial no difference in hemoglobin status between piglets receiving iron dextran and those receiving gleptoferron were observed.



Graph. 1 Effect of the Fe source on hemoglobin levels at three different timepoints; Source × Time, *P* = 0.031 SEM = 0.166

The absence of a growth difference between sources, despite the higher hemoglobin levels with gleptoferron, was expected, because body weight gain is an inaccurate indicator of iron status and a reduced gain occurs only under severe anemia (Ettle et al., 2008). In fact, in this trial even the piglets receiving iron dextran maintained a weaning hemoglobin level of 10.6 g/dL, above the anemic range,

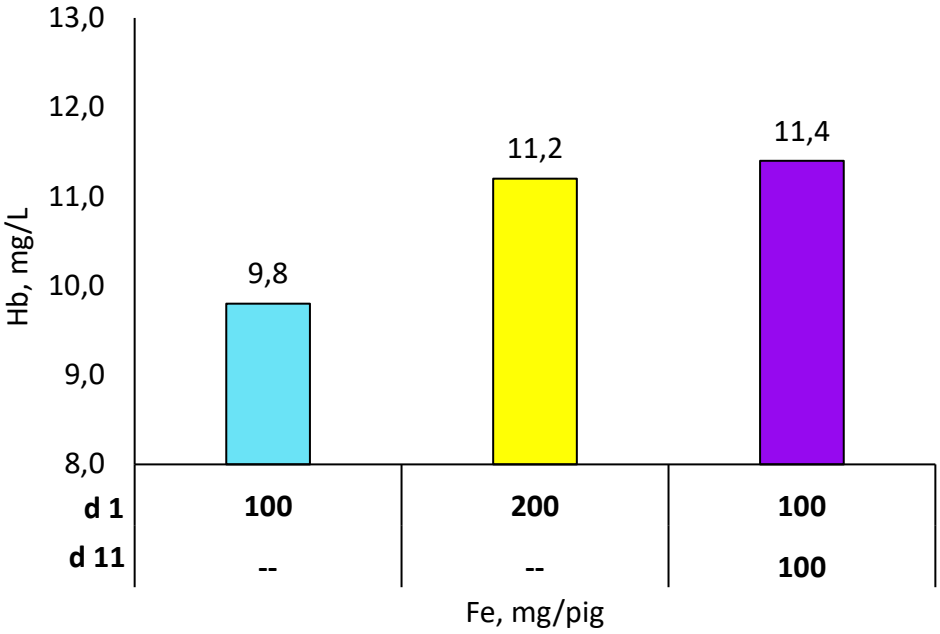
so the iron status of both groups was sufficient to support normal pre-weaning growth (Graph 2). The same result was reported by Morales et al., 2018, which found higher serum iron concentrations in piglets receiving iron from gleptoferron but no differences in growth performance between the two sources.



Graph. 2 Main effect of the Fe source on hemoglobin levels at weaning; *P = 0.001* SEM = 0.166

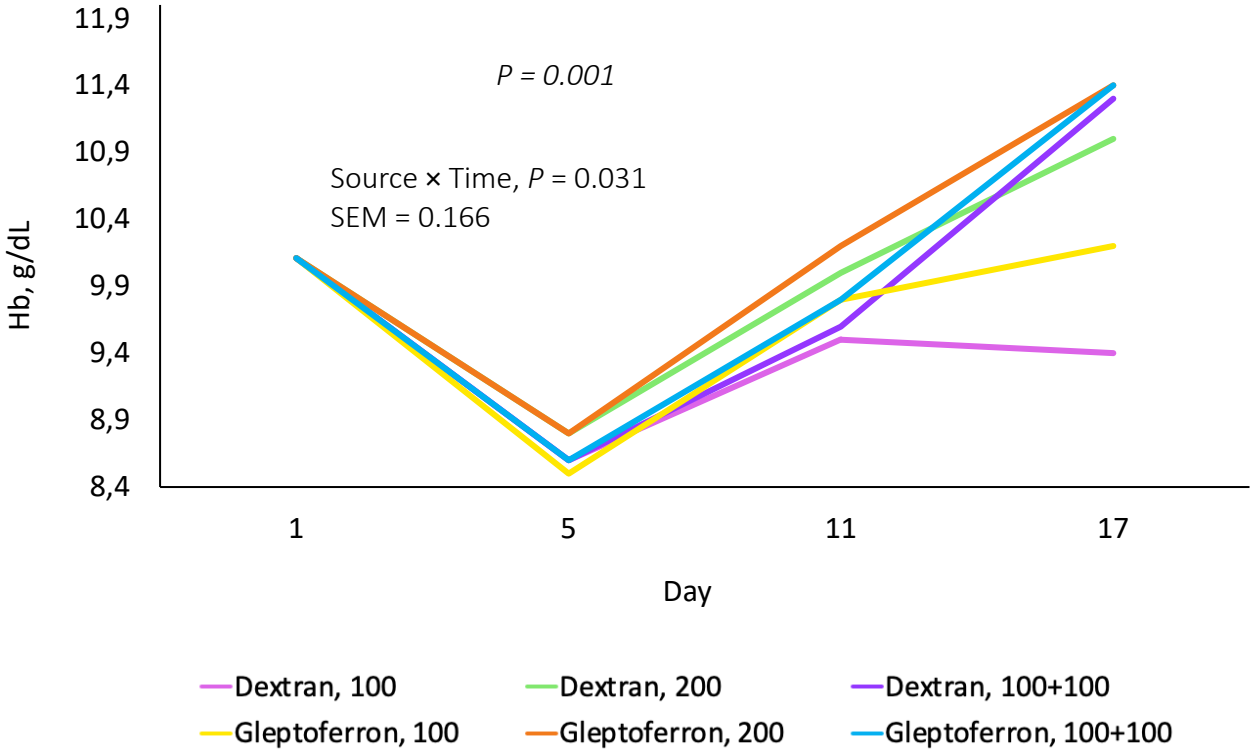
The administration program affected hemoglobin levels at weaning, with piglets receiving only 100 mg of iron on d 1 had the lowest value (9.8 g/dL), compared with pigs receiving 200 mg on d 1 (11.2 g/dL) or 100 mg on d 1 plus a second iron injection of 100 mg on d 11 (11.4 g/dL) (Graph 3). In agreement with those results, Williams et al., 2018 observed that weaning hemoglobin levels and blood iron status increased with dose and were maximized at 200 mg, with 100 mg producing lower weaning hemoglobin.

Based on the commonly used thresholds to identify anemia, a normal hemoglobin is above 11 g/dL, iron deficiency between 9 and 11 g/dL, and anemia at or below 9 g/dL (Morales et al., 2018). Piglets that received 100 mg of iron were characterized by iron deficiency, as their hemoglobin level of 9.8 g/dL is within the iron deficiency range, whereas piglets received 200 mg and split programs reached the normal range. The results are supported by Chevalier et al., 202 who found that only doses of 200 mg or more resulted in hemoglobin levels within the reference range to avoid anemia at weaning.



Graph 3. Main effect of the iron program if hemoglobin level at weaning (on day 17); *P < 0.001 a,b,c Differ, P< 0.05, SEM = 0.166*

How showed on graph 4, the split program reached the single 200 mg dose only at weaning because the second dose was given on d 11 and the effect in hemoglobin after an injection takes several days (Albers et al., 2022). The effect of the second injection on raising hemoglobin levels during lactation is confirmed by the literature (Friendship et al., 2021). In line with this, on day 11, the piglets from the split dose group had hemoglobin levels similar to the 100 mg group (9.7 vs. 9.6 g/dL) rather than the 200 mg group (10.1 g/dL). Their subsequent increase in hemoglobin supports the observation that a second iron injection has a greater effect when the initial iron status is low (Albers et al., 2022).



Graph 4. Hemoglobin levels during the lactation at four different timepoints. The purple and light blue lines in the graph represent the two split programs. It can be seen that on days 5 and 11, they show lower hemoglobin levels than green and orange lines (representing the single 200 mg injection programs), reaching their hemoglobin levels only at weaning (d 17). Program × Source × Time, P = 0.502; Program × Time, $P < 0.001$; Source × Time, $P = 0.031$; Program × Source, $P = 0.380$; Program, $P < 0.001$; Source, $P = 0.009$; Time, $P < 0.001$; SEM= 0.166

As the iron source, the program did not affect BW or ADG even though it changed hemoglobin. The fact that the piglets injected with only 100 mg of iron grew as well as piglets injected with 200 mg shows that 100 mg is enough for maximum pre-weaning growth, even though a higher dose is needed to improve iron levels in the blood (Williams et al., 2018). The split program raised weaning hemoglobin levels to the levels of a single 200 mg dose without improving growth, this agrees with some studies in which a second injection improved iron status but did not result in better growth performance (Williams et al., 2021). The same absence of a growth response to an additional injection, is reported from Albers et al. (2022) and Friendship et al. (2021).

Although the split dose offered no growth advantage over a single 200 mg injection, dividing the dose is a common practice because a single large parenteral dose in a newborn piglet, can induce oxidative stress and produce twice the hepatic oxidative DNA damage of a divided dose while both

corrected anemia (Lipiński et al., 2010). A high single dose also elevates plasma hepcidin, whereas the split protocol keeps hepcidin low while maintaining the same iron status (Starzyński et al., 2013). Thus, the split program tested matched the weaning hemoglobin of a single 200 mg dose, possibly with lower oxidative stress. However, the second dose involves double handling of the animals, leading to negative consequences for them and higher labor requirements.

Hemoglobin in the present study was measured with the HemoCue 201+ (HemoCue AB, Ängelholm, Sweden), this device was used to monitor iron status in comparable suckling-piglet studies such as Chevalier et al. (2023). Although some studies reported that this method can underestimate hemoglobin, the same device was used for all treatments, so any bias would have applied equally across groups and would not affect treatment comparisons (Almond et al., 2017). In addition, the HemoCue system has been validated for hemoglobin determination in pigs and showed high accuracy and a strong correlation (r = 0.98) with laboratory hematology analyzer (Kutter et al., 2012).

By 18 days after weaning, at d 35 of life, neither iron source nor administration program affected hemoglobin levels or ADG, this shows that the differences in iron levels seen during lactation disappear once the pigs entered the nursery. This agrees with other studies showing that pigs with low iron injections recover after they start consuming adequate dietary iron (Williams et al., 2018). A comparable result was reported by Chevalier et al. (2021) that shows that piglets receiving lower doses of iron were below the normal hemoglobin level, but reached it as they grew. This probably occurred because the piglets were close to iron deficiency, but not anemic at weaning. If they were anemic, probably their growth would have stayed lower for longer. Indeed, piglets that are anemic at weaning can result 0.82 kg lighter three weeks after weaning compared to piglets with normal hemoglobin levels (Perri et al., 2016).

As mentioned before, at approximately day 35 of life none of the three administration programs affected hemoglobin levels and BW. However, this result disagrees with the literature, in particular with a trial by Chevalier et al. (2023) in which a split program characterized by a second 200 mg iron dextran dose resulted at day 36 of life in a higher BW compared with the control group that just received 200 mg of iron in a single injection (10.51 vs. 10.85 kg). Furthermore, the split program group resulted in a higher hemoglobin level at around day 57 of life (10.51 vs. 10.85 g/dL). That difference is explained by the larger total dose of iron in that study (400 vs 200 mg), and the earlier timing of the second injection (days 6 - 8).

Overall, those results indicate that a single 100 mg injection on d 1 is sub optimal to support an adequate iron status at weaning and that delivering at least 200 mg of iron, either as a single dose or split between d 1 and d 11, is preferable and sufficient to prevent iron deficiency anemia.

10. Conclusions

In suckling piglets, the choice of injectable iron source and administration program can modify iron status but not growth, at least within the lactation and early post-weaning period. This difference means that decisions about the iron protocol should focus on keeping piglets healthy and preventing anemia at weaning and not on just on obtain higher body weight or daily growth. A single 100 mg dose at birth appears insufficient for this purpose, whereas at least 200 mg of iron, given in one injection or split between d 1 and d 11 of life, is a more effective choice, with gleptoferron providing a more favorable hemoglobin response than iron dextran. The fact that the differences in iron status disappeared early in the nursery without affecting performance, suggests that in healthy, non-anemic pigs, a higher or earlier iron dose builds larger iron reserves rather than increasing growth. The long-term benefits of these reserves, deserve further study.

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