

# An investigation of iron deficiency and anemia in piglets and the effect of iron status at weaning on post-weaning performance

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## Summary

**Objectives:** To determine iron status of pigs at weaning and its effects on post-weaning performance, and to determine whether high concentrations of zinc oxide (ZnO) in feed are associated with postweaning anemia.

**Materials and methods:** A small, medium, and large piglet (N = 1095) were selected per litter 1 to 2 days before weaning from 20 Ontario (Canada) swine farms. Serum and whole blood samples and body weights were collected. Three weeks later, a second body weight and blood sample were collected from the same pigs. Hemoglobin (Hb) and other blood parameters were analyzed to

assess iron status and associations with post-weaning performance. Iron supplementation protocols and ZnO concentrations in nursery feed were collected.

**Results:** Anemic and iron-deficient pigs presented at weaning on most participating farms. Pigs that had been anemic at weaning were 0.82 kg lighter 3 weeks post weaning than piglets that had normal Hb values at weaning ( $P < .05$ ). Larger piglets at weaning had lower red cell parameters and serum iron, and higher total iron binding capacity, than smaller piglets (all  $P < .05$ ). More pigs were anemic 3 weeks post weaning than at weaning ( $P < .05$ ), and prevalence of anemia

was associated with high ZnO concentrations ( $P < .05$ ).

**Implications:** Iron supplementation protocols used in the study herds were inadequate to prevent iron deficiency, particularly in the largest pigs. Anemic pigs at weaning have slower growth rates in the nursery. Consumption of nursery starter feeds containing high concentrations of ZnO is associated with post-weaning anemia.

**Keywords:** swine, anemia, iron, nursery performance, hemoglobin

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## Resumen - Una investigación de la deficiencia de hierro y anemia en lechones y el efecto del estado de hierro en el desempeño post destete

**Objetivos:** Determinar el estatus de hierro en cerdos al destete y sus efectos en el desempeño post destete, y determinar si las altas concentraciones de óxido de zinc (ZnO) en el alimento están relacionadas con la anemia post destete.

**Materiales y métodos:** Se seleccionó un lechón pequeño, mediano y grande (N = 1095) por camada 1 a 2 días antes del destete de 20 granjas porcinas de Ontario (Canadá). Se recolectaron suero, muestras

de sangre completa y pesos corporales. Tres semanas después, se tomó un segundo peso corporal y muestra de sangre de los mismos cerdos. Se analizaron la hemoglobina (Hb) y otros parámetros de sangre para evaluar el nivel de hierro y las asociaciones con el desempeño post destete. Se recolectaron los protocolos de suplementación de hierro y las concentraciones de ZnO en el alimento de área de destete.

**Resultados:** Se encontraron cerdos deficientes en hierro y anémicos al destete en la mayoría de las granjas participantes. Los cerdos que habían estado anémicos al destete fueron 0.82 kg más ligeros 3 semanas post destete que los lechones que tuvieron valores de Hb

normales al destete ( $P < .05$ ). Los lechones más grandes al destete presentaron hierro de suero y parámetros de células rojas, más bajos, y una capacidad de unión de hierro total más alta que lechones más pequeños (todos  $P < .05$ ). Hubo más cerdos anémicos 3 semanas post destete que al destete ( $P < .05$ ), y la prevalencia de anemia se asoció con altas concentraciones de ZnO ( $P < .05$ ).

**Implicaciones:** Los protocolos de suplementación de hierro utilizados en los hatos de estudio fueron inadecuados para prevenir la deficiencia de hierro, particularmente en los cerdos más grandes. Los cerdos anémicos al destete tuvieron índices de crecimiento más lentos en el área de destete. El consumo de alimentos iniciadores en el área de destete que contenían altas concentraciones de ZnO se asocia con la anemia post destete.

**Résumé - Étude sur la déficience en fer et l'anémie chez les porcelets et les effets du statut en fer au sevrage sur les performances post-sevrage**

**Objectifs:** Déterminer le statut en fer de porcs au sevrage et les effets sur les

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performances post-sevrage, et déterminer si des concentrations élevées en oxyde de zinc (ZnO) dans la ration sont associées à de l'anémie en post-sevrage.

**Matériels et méthodes:** Un porcelet de petite, moyenne, et grande taille (N = 1095) ont été sélectionnés par portée 1 à 2 jours avant le sevrage de 20 fermes porcines en Ontario (Canada). Des échantillons de sérum et de sang entier ont été obtenus et le poids corporel noté. Trois semaines plus tard, à partir des mêmes animaux, on procéda à une deuxième série de prélèvements sanguins et de prise de poids. Le taux d'hémoglobine (Hb) et d'autres paramètres sanguins ont été analysés pour évaluer le statut en fer et les associations avec les performances post-sevrage. Les protocoles de supplémentation en fer et les concentrations de ZnO dans les rations en pouponnière ont été obtenus.

**Résultats:** Des porcs anémiques et avec des déficiences en fer au moment du sevrage étaient présents dans la plupart des fermes participantes. Les porcs qui avaient été anémiques au sevrage étaient plus léger de 0,82 kg 3 semaines post-sevrage que des porcelets qui avaient des valeurs normales de Hb au sevrage ( $P < 0,05$ ). Les porcelets plus gros au sevrage avaient des paramètres érythrocytaires et une quantité de fer sérique inférieurs, et une capacité de liaison du fer total plus élevée que les plus petits porcelets (tous les  $P < 0,05$ ). Plus de porcs étaient anémiques 3 semaines post-sevrage qu'au moment du sevrage ( $P < 0,05$ ), et la prévalence d'anémie était associée avec des concentrations élevées de ZnO ( $P < 0,05$ ).

**Implications:** Les protocoles de supplémentation en fer utilisés dans les troupeaux étudiés étaient inadéquats pour prévenir une déficience en fer, particulièrement chez les porcs plus gros. Les porcs anémiques au sevrage avaient des taux de croissance plus lents dans la pouponnière. La consommation de rations en pouponnière contenant des concentrations élevées en ZnO est associée à de l'anémie en post-sevrage.

It is well established that insufficient intake of iron in suckling pigs results in iron deficiency or anemia, where the concentration of hemoglobin (Hb) and the number and size of red blood cells (RBCs) decline below the normal range.<sup>1</sup> The suckling pig, regardless of breed, is susceptible to iron deficiency, anemia, or both.<sup>2</sup> The pig is born with limited iron, having a total body store of approximately 50 mg of iron, mostly

incorporated in hemoglobin.<sup>3</sup> Sow milk is a poor source of iron, providing piglets with only 1 mg of iron a day.<sup>3</sup> Pigs lack access to soil, a rich source of iron,<sup>3</sup> due to confinement rearing indoors, and the modern pig has been selected for rapid growth. In the first week of life, piglets double their weight and increase their plasma volume by 30%, thereby diluting the concentration of Hb.<sup>4</sup> The daily iron requirement for piglets is approximately 7 mg, and, therefore, the limited iron that piglets are born with is inadequate in preventing iron deficiency and anemia, since these body stores dilute very rapidly.<sup>3,5-8</sup>

Piglets require exogenous iron supplementation within the first week of life to compensate for their limited iron and to prevent iron deficiency and anemia. It is commonly recommended to administer a 200-mg intramuscular (IM) injection of iron dextran within the first 3 days of life. Although oral iron is sometimes used, parenteral administration of iron is the most common method of iron supplementation for pigs on commercial swine farms.<sup>9,10</sup> A Hb concentration below 110 g per L is indicative of iron deficiency, and a Hb concentration below 90 g per L is indicative of anemia.<sup>11,12</sup> Iron deficiency occurs when there is a reduction (or usage without replacement) in the total content of iron in the body.<sup>13</sup> When there is a lack of iron in the body, nutrient requirements are not met. During the early stages, clinical signs such as anemia may not be apparent, whereas anemia occurs when iron deficiency is severe and causes a reduction in erythropoiesis.<sup>13</sup>

Iron supplementation is performed on a routine basis on commercial farms; however, the iron status of piglets is seldom evaluated. With updated management practices and modern genetic lines, sows farrow larger litters and piglets grow at an even greater rate than in previous decades.<sup>14,15</sup> Therefore, it is imperative to reassess whether the routine iron supplementation protocols used today on commercial swine farms are still adequate to prevent iron deficiency and anemia in modern piglets. This could have animal-health and economic implications, as piglets that have inadequate iron stores may develop a suppressed immune system, resulting in an impaired ability to resist infectious and parasitic diseases, and have a slower growth rate and increased morbidity and mortality.<sup>9</sup>

Iron deficiency, anemia, or both may occur on commercial swine farms because of husbandry errors, ie, inadequate dosing or timing of administration, or it may be that modern piglets require a higher dosage of iron during routine iron supplementation procedures. Also, common management practices, such as use of high concentrations of zinc oxide in the feed (> 2000 mg per kg) to control *Escherichia coli* diarrhea in newly weaned pigs, may decrease iron absorption from the feed. Copper, iron, and zinc are trace minerals that have similar physical and chemical properties. When there is an imbalance in one of these minerals, there may be an antagonistic effect on the nutritional availability of another mineral.<sup>16</sup> Thus, the use of high concentrations of zinc oxide in feed (> 500 mg per kg) may alter absorption of iron.

The objectives of this epidemiological study were to determine if iron deficiencies or anemia are present in pigs at weaning and if they affect post-weaning performance, and to determine if iron deficiency or anemia persists in the nursery stage and, if so, whether high concentrations of zinc oxide added to starter feeds is associated with the occurrence of post-weaning anemia.

## Materials and methods

The Animal Care Committee at the University of Guelph, which follows the guidelines of the Canadian Council for Animal Care, reviewed and approved this study.

## Study design and sampling

Twenty swine farms from 10 counties across southern Ontario were enrolled. The farms were sampled to represent a wide variety of production types, management practices, and sow-herd sizes. A questionnaire was administered at each farm to collect information regarding iron supplementation practices, including the age of piglet at the time of administration, dose, and type of iron supplementation product(s) used. The questionnaire also captured farm-management information such as the size of the sow herd, weaning age, and pig flow.

Each farm was visited twice. At the first visit, 1 to 2 days prior to weaning, litters were systematically selected starting at the first crate in the farrowing room until a maximum of 20 litters per farm was reached. Selection was based on visual assessment of three piglets per litter, including one large,

one medium, and one small piglet. Pigs were excluded if they exhibited physical abnormalities such as an abscess or hernia, or if they were lame or unthrifty, ie, thin body condition. Piglets were selected in this manner to obtain a balanced sample of different-sized piglets to enable assessment of iron status by body size. Each selected piglet was individually ear tagged and weighed. Blood samples were collected from each piglet via the orbital sinus technique using a Monoject standard hypodermic needle, 16-gauge  $\times$  1" (Covidien, Mansfield, Massachusetts). Blood was collected in 8.5-mL tubes (BD Vacutainer; BD, Franklin Lakes, New Jersey), and whole blood samples were collected in 6-mL tubes containing ethylenediamine tetraacetic acid (EDTA) (BD Vacutainer; BD). At the second visit, 3 weeks after the first visit, the same pigs were weighed and whole blood samples were collected using the techniques described. In order to evaluate the prevalence of iron deficiency and anemia in piglets at weaning, a classification for Hb status was determined a priori, based on current classifications found in the literature. Normal iron status was defined as a Hb value of  $> 110$  g per L, iron deficiency was defined as a Hb value of  $> 90$  g per L but  $\leq 110$  g per L, and anemia was defined as a Hb value of  $\leq 90$  g per L.<sup>11,12</sup> The prevalence of iron deficiency and anemia in piglets was determined for each farm.

### Hemoglobin measurement

Hemoglobin values were analyzed using two methods. On the initial sampling, 1 to 2 days prior to weaning, the whole blood samples collected from each of the piglets were analysed at the Animal Health Laboratory (AHL) at the University of Guelph using the ADVIA 2120 per 2120i Hematology system (Siemens Healthcare Diagnostics, Deerfield, Illinois) as per standard protocols. Briefly, the blood sample and the ADVIA 2120 HGB reagent are mixed together in the Hb chamber of a colorimeter. The Hb reaction involves two steps: RBCs are lysed to release Hb, and heme iron found in Hb is oxidized from the ferrous to the ferric state, and then combined with cyanide in the ADVIA 2120 HGB reagent to form the product.<sup>17</sup> Optical readings are obtained colorimetrically at 546 nm.<sup>17</sup>

The second Hb measurement occurred 3 weeks after the initial visit, when the pigs were in the nursery. At this time, Hb in whole blood samples was measured using an

automated hematology handheld instrument (STAT-Site M Hgb meter, Boerne, Texas). The STAT-Site M Hgb meter contains a plastic card with reagent pads for determining the concentration of Hb. This device provides measurements of blood Hb content within 30 seconds after 15  $\mu$ L of blood has been applied to the test strip. The amount of color produced from azide-methemoglobin is proportional to the concentration of Hb in the sample.<sup>18</sup>

### Hematology measurements

The red blood cell (RBC) count, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) in whole blood samples collected at the first visit were analyzed by the AHL using the ADVIA 2120 per 2120i Hematology System (Siemens Healthcare Diagnostics). All of these indicators of iron status were analyzed from a single optical cytometer after dilution of the samples with ADIVA 2120 RBC reagent. As the reaction mixture moves towards the flowcell in the optical cytometer, a laser strikes the cells and generates electronic scatter signals to measure the size, volume, and internal characteristics of the cells.

To determine serum iron and total iron binding capacity (TIBC), the same whole blood samples were also analyzed by the AHL using a Roche per Hitachi cobas 6000 c501 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Briefly, iron is released from transferrin under acidic conditions. Ascorbate decreases the number of released  $\text{Fe}^{+++}$  ions to  $\text{Fe}^{++}$  ions, which then react with the iron reagent ferrozine.<sup>17</sup> This reaction forms a colored complex in which TIBC is measured photometrically and is proportional to the color intensity.<sup>13</sup>

### Zinc oxide levels in the feed

Feed tags were collected at each farm to evaluate the concentration of zinc oxide in the first-phase nursery diets. The Canadian Food Inspection Agency (CFIA) and Feeds Regulations (1983) state that the maximum zinc oxide concentration in feed is 500 mg per kg for nursery pigs, without a request from the producer to increase the concentration.<sup>19</sup> The CFIA also states that the actual amount of zinc oxide in mg per kg of the feed must be present on the label. All participating farms fed diets meeting daily zinc requirements and

higher. Zinc oxide concentration in the feed was categorized: nutritional dose ( $\leq 500$  mg per kg), high dose (2000 to 3000 mg per kg), and very high dose ( $> 3000$  mg per kg).

### Statistical analysis

All data were entered into an Excel spreadsheet (Microsoft Corporation, Redmond, Washington) and then imported into Stata 12 Intercooled for Windows XP (Statacorp LP, College Station, Texas) for analyses. The association between Hb status (g per L) at weaning and subsequent nursery growth performance (measured as the 3-weeks-post-weaning weight) was analyzed using mixed linear regression. The dependent variable in the model was the 3-weeks-post-weaning weight. Extraneous variables that were used in the final model include parity of the sow, age at weaning, weaning weight, and Hb status at weaning. The model was built using forward stepwise criteria and designed parsimoniously. Hemoglobin status was categorized as follows: normal Hb ( $> 110$  g per L), iron deficient ( $> 90$  g per L but  $\leq 110$  g per L) or anemic ( $\leq 90$  g per L). Dam (sow) parity was categorized as parity 1 (gilt), parity 2 to 5, and parity  $> 5$ . The age of the piglet when the iron supplementation was administered was categorized as  $\leq 1$  day of age, 2 to 4 days of age, and 5 to 7 days of age.

All extraneous variables were initially screened for univariable associations using linear regression and considering farm as a random effect. Univariable associations with a liberal  $P$  value of  $< .2$  were considered for the final model. Linearity of continuous predictor variables with 3-weeks-post-weaning weight as the outcome variable was assessed using two methods: visually using a lowess smoother (smoothed locally weighted scatter plot), and using a quadratic term. Confounding was assessed throughout model building. A confounding variable was defined as a variable whose removal from the model changed the coefficient of any predictor variable by greater than 20%. Two-way interactions were generated between all extraneous variables in the initial model and were included in the final model only if they were statistically significant ( $P < .05$ ). Extraneous variables were included in the final model if variables had a  $P < .2$  in univariable analysis and then a  $P < .05$  in the final model, if the variable was a confounder, or if the variable was part of a statistically significant interaction ( $P < .05$ ). These variables were then assessed for collinearity

using Pearson correlation analysis. Potential outliers, influential points, and the model assumptions were assessed graphically.

To assess the association between various iron indicators and weaning weight (category), eight separate linear mixed models were created. Each iron indicator (RBC, Hb, HCT, MCV, MCH, MCHC, serum iron, and TIBC) was modeled separately as the dependent variable and in each of the models, the association between the size of the piglet at weaning (small, medium, or large) and each iron indicator was evaluated. In these models, sex of the piglet, parity of the dam, age at weaning, weaning weight, and the type of iron administered were modeled as fixed effects, and farm was modeled as a random effect using the methods described for analysis involving Hb.

To explore the association between the zinc oxide content in feed and anemia at 3 weeks post weaning, a mixed logistic regression model was built. For this model, iron status was dichotomized as anemic if the piglet had a Hb concentration  $\leq 90$  g per L or normal if the piglet had a Hb concentration  $> 90$  g per L. Iron status at weaning, the type of iron administered, and age at weaning were modeled as fixed effects. Zinc oxide concentration in the feed was categorized as nutritional dose, high dose, or very high dose, as described. Confounding was assessed throughout model building as described. Potential outliers and influential points were evaluated graphically, and model diagnostics were performed.

## Results

The farms enrolled varied in size from 112 to 1500 sows, with the majority of farms being farrow-to-finish and four being farrow-to-wean. For the farrow-to-wean farms, the initial sampling visit occurred at each of the four sites, and then the second sampling visit occurred at each of the farms' off-site nurseries. A total of 1095 pigs were sampled, with a range of 13 to 20 litters per farm. All male piglets enrolled in this study had been castrated. Farm-specific demographics, iron supplementation protocols, and mean growth performance values, including piglet weaning weight, 3-weeks-post-weaning weight, and average daily gain (ADG) for each farm, are presented in Table 1. Of the 20 farms sampled, 60% (12 of 20) mixed their iron product with other pharmaceutical products such as meloxicam or penicillin.

The mean age ( $\pm$  standard deviation) at which piglets were initially sampled (1 to

2 days prior to weaning) was  $21.8 \pm 4.2$  days. The mean weight of pigs at initial sampling (1 to 2 days prior to weaning) was  $6.4 \pm 1.8$  kg. The mean weight of pigs in the small ( $n = 365$ ), medium ( $n = 365$ ), and large ( $n = 365$ ) weight categories were  $5.2 \pm 1.5$  kg,  $6.5 \pm 1.4$  kg, and  $7.5 \pm 1.6$  kg, respectively. The prevalence of iron deficiency and anemia at 1 to 2 days prior to weaning and at 3 weeks post weaning for individual farms are presented in Table 1. The within-herd prevalence of iron deficiency and anemia at weaning ranged between 0% and 61% and 0% and 46%, respectively. The between-herd prevalences of iron deficiency and anemia at weaning were 28% and 6%, respectively. Nineteen of the 20 farms (95%) had piglets with low Hb values (iron deficient or anemic or both) at weaning. Upon sampling at 3 weeks post weaning, the within-herd prevalence for iron deficiency and anemia ranged between 29% and 74% and 6% and 32%, respectively. The between-herd prevalences of iron deficiency and anemia at this time were 43% and 18%, respectively. From the initial sampling day to 3 weeks post weaning, 72 pigs (6.6%) were lost from the study. The reasons for piglet loss were not recorded, but pig loss was evenly distributed among the farms. Of the 72 pigs that were missing, 58% had normal Hb values, 35% were iron deficient, and 7% were anemic at weaning.

The associations between piglet weight categories at weaning and iron indicators are presented in Table 2. Medium-sized piglets at weaning had a Hb concentration 2.7 g per L higher than larger pigs at weaning ( $P < .01$ ). Smaller-sized pigs at weaning had a Hb concentration 3.4 g per L greater than large pigs at weaning ( $P < .001$ ). Hemoglobin status at weaning did not differ between small and medium weight categories.

The eight models illustrating the associations between various iron indicators with body-weight category at weaning are presented in Table 3. The mean values for various iron indicators, by piglet weight category, can be found in Table 4. Piglets from the large weight category had lower Hb, serum iron, HCT, MCV, MCH, and MCHC values than did piglets in the small and medium weight categories ( $P < .05$ ). Total iron binding capacity values were higher in the large-sized piglets than in the small and medium-sized piglets ( $P < .01$ ). There was no statistical difference found between each of the weight categories and RBC counts.

The final model illustrating the association between Hb status at weaning and 3-weeks-post-weaning weight is presented in Table 4. No significant interactions or confounders were identified. The final model revealed that anemic piglets at weaning had a 0.82 kg lower 3-weeks-post-weaning weight than did piglets with normal Hb values at weaning ( $P < .01$ ). Also, anemic pigs at weaning were on average 0.69 kg lighter in weight at 3 weeks post weaning than pigs that were classified as iron deficient at weaning ( $P < .05$ ). There was no statistical difference in 3-weeks-post-weaning weight when comparing iron-deficient pigs at weaning with pigs with a normal Hb status. Piglets from sows whose parities ranged from 2 to 5 and from sows of parities  $> 5$  had higher 3-weeks-post-weaning weights than did piglets from gilts ( $P < .05$ ). There was no difference in the 3-weeks-post-weaning weights between piglets administered an iron dextran injection or a gleptoferron injection. Piglets weaned at an older age had a 0.12-kg higher weight at 3 weeks post weaning ( $P < .001$ ).

The zinc oxide content in feed, collected from feed tags, ranged between 250 and 7000 mg per kg on the farms. The logistic regression model created to explore the association between the zinc oxide content in feed and anemia at 3 weeks post weaning is presented in Table 5. The odds of nursery pigs being anemic was 3.4 times greater for pigs consuming high doses of zinc oxide in feed than in those consuming a nutritional dose of zinc oxide in feed ( $P < .05$ ). The odds of nursery pigs being anemic was 4.1 times greater for those consuming starter feeds containing very high concentrations of zinc oxide than for those consuming a nutritional dose of zinc oxide ( $P < .05$ ). There was no difference in the odds of anemia in pigs fed very high doses of zinc oxide, compared to pigs fed a high dose of zinc oxide ( $P > .05$ ). The type of iron administered was a confounder in this model. This is likely because anemia was classified differently (dichotomized) in this model, but was classified categorically in the main model, and because the majority of farms used iron dextran, hence this variable was included in the model.

## Discussion

Despite routine iron supplementation during the first week of life, pigs with low Hb values were identified at weaning on almost all farms, and, surprisingly, the prevalence of anemic pigs was greater 3 weeks after weaning.

**Table 1:** Summary of farm production parameters, iron supplementation protocols, and iron status of piglets from 20 commercial swine farms in Ontario (Canada) at weaning and 3 weeks post weaning\*

Farm (no. sows)	No. pigs at weaning (no. litters)	Age (days)†		Mean weight (kg)‡			Iron status§						ADG (kg)
		Iron	Weaning	Weaning	Post-weaning	% at weaning (n)			% 3 weeks post weaning (n)				
						Normal	Deficient	Anemic	Normal	Deficient	Anemic		
1 (600)	57 (19)	< 1	21.4 (± 2.2)	6.7 (± 1.7)	12.0 (± 2.9)	35 (20)	61 (35)	4 (2)	63 (35)	30 (17)	7 (4)	0.25 (± 0.06)	
2 (1400)	60 (20)	2-4	19.2 (± 0.9)	6.3 (± 1.4)	13.4 (± 2.4)	63 (38)	37 (22)	0 (0)	ND	ND	ND	0.34 (± 0.08)	
3 (500)	60 (20)	5-7	27.9 (± 2.8)	8.9 (± 1.9)	17.4 (± 2.9)	72 (43)	27 (16)	1 (1)	62 (37)	28 (17)	10 (6)	0.40 (± 0.09)	
4 (300)	60 (20)	5-7	20.9 (± 1.7)	5.7 (± 1.3)	11.5 (± 2.3)	80 (48)	12 (7)	8 (5)	20 (12)	57 (34)	23 (14)	0.27 (± 0.06)	
5 (250)	51 (17)	5-7	24.5 (± 1.3)	6.6 (± 1.2)	12.7 (± 2.7)	37 (19)	39 (20)	24 (12)	19 (9)	75 (35)	6 (3)	0.29 (± 0.09)	
6 (112)	48 (16)	5-7	26.2 (± 2.7)	6.4 (± 1.4)	11.5 (± 3.1)	6 (3)	48 (23)	46 (22)	28 (13)	64 (30)	8 (4)	0.23 (± 0.09)	
7 (1000)	60 (20)	< 1	21.0 (± 1.7)	6.5 (± 1.4)	12.6 (± 1.9)	30 (18)	58 (35)	12 (7)	59 (35)	29 (17)	12 (7)	0.29 (± 0.06)	
8 (850)	60 (20)	2-4	18.7 (± 1.3)	5.9 (± 1.4)	9.3 (± 2.6)	70 (42)	25 (15)	5 (3)	35 (21)	33 (20)	32 (19)	0.16 (± 0.08)	
9 (140)	39 (13)	< 1	29.5 (± 3.2)	5.2 (± 1.3)	7.3 (± 1.7)	59 (23)	33 (13)	8 (3)	26 (10)	44 (17)	30 (12)	0.10 (± 0.06)	
10 (1250)	60 (20)	2-4	25.3 (± 1.6)	7.2 (± 1.5)	14.4 (± 2.8)	83 (50)	17 (10)	0 (0)	27 (16)	42 (25)	31 (18)	0.36 (± 0.08)	
11 (130)	39 (13)	2-4	25.5 (± 3.5)	7.7 (± 1.8)	14.5 (± 3.2)	100 (39)	0 (0)	0 (0)	28 (11)	46 (18)	26 (10)	0.31 (± 0.16)	
12 (640)	60 (20)	2-4	17.5 (± 1.0)	6.0 (± 1.2)	11.0 (± 2.2)	80 (48)	20 (12)	0 (0)	13 (8)	51 (30)	36 (21)	0.24 (± 0.08)	

Table 1 continued on page 15

Different reference limits are reported in the literature regarding how low Hb concentration can be before anemia is diagnosed.<sup>10-12</sup> Wei et al<sup>10</sup> suggest that a Hb concentration above 100 g per L is considered normal and that a Hb concentration below 60 g per L indicates severe anemia. Anemia has also been defined as a Hb concentration below 80 g per L.<sup>9</sup> A recent paper by Bhattarai and Nielsen<sup>12</sup> used a Hb concentration below 110 g per L as indicative of iron deficiency and a Hb concentration below 90 g per L as indicative of anemia. Being able to distinguish low iron prior to evidence of clinical anemia is a useful concept for practitioners who are monitoring the effectiveness of an iron supplementation program, and for this reason we chose to use the Bhattarai and Nielsen<sup>12</sup> categories to assess Hb concentrations. Since a slightly higher threshold

was chosen for defining iron deficiency and anemia, compared to that in other published material, this may partly be the reason why the prevalences were high.

Hemoglobin is commonly used as a measurement of iron status, because 80% to 90% of the iron present in the suckling piglet is used in forming Hb.<sup>20</sup> Hemoglobin is an important protein involved in cellular metabolism, as it transports oxygen from the lungs to other body tissues, and transports carbon dioxide back to the lungs for expulsion via the respiratory tract.<sup>4,14</sup> The greater the concentration of Hb per given unit of blood, the greater the amount of oxygen that can be carried in blood.<sup>21</sup> Iron deficient and anemic pigs have fewer RBCs containing less Hb, compared to piglets with normal Hb levels.<sup>4</sup> Along with Hb, other iron indicators, such as serum iron levels and TIBC,

are also important for assessing iron status in swine. Serum iron measures the amount of iron circulating in the blood bound to transferrin, an important protein that binds and transports iron in blood. Total iron binding capacity measures the blood's capacity to bind with transferrin.

Although Hb status is the most frequently used parameter for evaluating iron deficiency and anemia in swine, it is possible that other blood parameters may be more sensitive in detecting the early stages of iron deficiency.<sup>22</sup> For instance, Bhattarai and Nielsen<sup>12</sup> were not able to find a difference in hemoglobin concentration between various piglet sizes, but found that large pigs had lower serum iron and higher TIBC than other pigs, indicating that iron is utilized faster in bigger piglets, making them prone to iron deficiency. Bhattarai and Nielsen<sup>12</sup>

**Table 1 continued**

Farm (no. sows)	No. pigs at weaning (no. litters)	Age (days)†		Mean weight (kg)‡			Iron status§						ADG (kg)
		Iron	Weaning	Weaning	Post-weaning	% at weaning (n)			% 3 weeks post weaning (n)				
						Normal	Deficient	Anemic	Normal	Deficient	Anemic		
13 (1500)	60 (20)	2-4	19.9 (± 0.7)	5.6 (± 1.4)	11.1 (± 2.1)	97 (58)	3 (2)	0 (0)	28 (17)	45 (27)	27 (16)	0.26 (± 0.05)	
14 (600)	57 (19)	2-4	17.1 (± 1.3)	5.2 (± 1.2)	8.6 (± 1.6)	77 (44)	23 (13)	0 (0)	51 (29)	37 (21)	12 (7)	0.17 (± 0.04)	
15 (535)	60 (20)	< 1	21.1 (± 2.7)	6.8 (± 1.7)	13.3 (± 3.0)	70 (42)	30 (18)	0 (0)	48 (29)	32 (19)	20 (12)	0.22 (± 0.06)	
16 (250)	60 (20)	< 1	18.0 (± 1.7)	5.1 (± 1.2)	8.7 (± 2.1)	92 (55)	8 (5)	0 (0)	34 (20)	53 (32)	13 (8)	0.16 (± 0.06)	
17 (85)	42 (14)	2-4	18.3 (± 4.3)	5.3 (± 1.5)	12.8 (± 3.4)	48 (20)	50 (21)	2 (1)	37 (15)	49 (20)	14 (6)	0.36 (± 0.10)	
18 (420)	60 (20)	2-4	20.8 (± 1.4)	6.4 (± 1.4)	11.2 (± 1.7)	98 (59)	2 (1)	0 (0)	40 (24)	35 (21)	25 (15)	0.23 (± 0.06)	
19 (262)	42 (14)	2-4	21.7 (± 4.0)	6.0 (± 1.2)	11.6 (± 2.1)	31 (13)	52 (22)	17 (7)	37 (15)	56 (23)	7 (3)	0.26 (± 0.07)	
20 (650)	60 (20)	5-7	26.4 (± 2.8)	8.4 (± 1.5)	14.8 (± 2.4)	77 (46)	23 (14)	0 (0)	75 (44)	24 (14)	1 (1)	0.30 (± 0.07)	
<b>Total (573)</b>	<b>54.8 (18.2)</b>	<b>NA</b>	<b>21.8 (± 2.1)</b>	<b>6.4 (± 1.4)</b>	<b>12.0 (± 2.4)</b>	<b>65.2 (728)</b>	<b>28.4 (304)</b>	<b>6.4 (63)</b>	<b>38.3 (400)</b>	<b>43.7 (437)</b>	<b>18.0 (186)</b>	<b>0.26 (± 0.07)</b>	

\* Farms 2, 7, 12, and 13 were farrow-to-wean farms; all other were farrow-to-finish farms. Farms 1, 6, 8, 14, and 20 used a gleptoferron product as an iron supplement; all other farms used an iron dextran product.

† Age at iron administration and mean age at weaning (± SD).

‡ Body weight (mean ± SD) at weaning and 3 weeks post weaning; ADG calculated by (3-weeks-post-weaning weight – weaning weight) ÷ no. of days between visits.

§ Normal, hemoglobin (Hb) > 110 g/L; iron deficient, Hb ≤ 110 g/L but > 90 g/L; anemic, Hb ≤ 90 g/L. At weaning, Hb was measured at the Animal Health Laboratory; at 3 weeks post weaning, Hb was measured using a hand-held meter (STAT-Site M Hgb meter; Boerne, Texas). SD = standard deviation; ADG = average daily gain; NA = not applicable; ND = not done (lost samples).

also concluded that using Hb as a diagnostic tool may underestimate the iron requirements for young growing piglets. With this in mind, additional iron indicators were analyzed in this study. The results for serum iron, HCT, MCV, MCH, and MCHC in each pig weight category at weaning agreed with the results of the Hb measurements for assessing iron status in this study. Because Hb can be easily and inexpensively measured using hand-held instruments that can be used on farm, these results support the continued use of Hb to monitor iron status on pig farms.

All of the measurements for iron status used in the present study indicate that the larger piglets at weaning were more likely to be iron deficient than were the small and medium-sized piglets. The data from the current study also indicate that, on most farms, the traditional supplementation of 200 mg of parenteral iron is insufficient to meet the needs of the large and fast-growing piglets,

and a higher dosage of iron or a second injection of iron at a later date during the suckling period may be required.

The prevalence of anemia and iron deficiency in pigs at weaning found in the current study is similar to the results from recent studies in various countries.<sup>11</sup> Walsh et al<sup>23</sup> found 30% of Ontario pigs to be anemic at weaning, when assessing Hb status on a single commercial swine farm. The current study confirms that identifying iron-deficient or anemic piglets at weaning is not uncommon on Ontario commercial pig farms. There are other possible reasons, in addition to greater nutritional requirements for fast growing pigs, which might explain why anemic and iron-deficient piglets are present at weaning. One possible reason is human error during administration of the iron supplementation, eg, some piglets are missed during the process or iron is given

late. It is also possible that there could be injection-site leakage resulting in dose variation when iron is administered during processing, with some pigs thereby more at risk for anemia because they did not receive a full dose of iron product. In this study, on 60% of the participating farms, an iron product was used that had been mixed with penicillin or meloxicam. This is surprising because “mixing two or more medications in a syringe for delivery to animals is a form of compounding and is not permitted” according to the Canadian Quality Assurance program (CQA Producer Manual, Version 2.1, D4-6; 2007)<sup>24</sup> and Health Canada’s policy on drug compounding in human and veterinary medicine (Policy on Manufacturing and Compounding Drug Products in Canada POL-0051; 2009).<sup>25</sup> To the authors’ knowledge, it is unknown what effect, if any, compounding pharmaceuticals with iron products may have on the uptake

**Table 2:** Eight individual models illustrating the associations between various iron-status indicators and the body-weight category 1 to 2 days prior to weaning among 1095 piglets from 20 Ontario (Canada) commercial swine farms

Model*	Weight category†	Coefficient‡	SE	95% CI	P	Contrast medium versus small pigs coefficient	SE	95% CI	P
Hb (g/L)	Small	3.43	0.923	1.625, 5.243	< .001	-.734	0.923	-2.543, 1.076	.43
	Medium	2.70	0.923	0.891, 4.510	< .001				
Serum iron (umol/L)	Small	6.95	0.820	5.345, 8.560	< .001	-3.540	0.820	-5.149, 1.933	< .001
	Medium	3.41	0.820	1.803, 5.019	< .001				
Hematocrit (L/L)	Small	0.01	0.003	0.001, 0.013	.02	0.000	0.003	-0.006, 0.006	.98
	Medium	0.01	0.003	0.001, 0.013	.02				
Mean corpuscular volume (fL)	Small	2.25	0.371	1.521, 2.974	< .001	-0.969	0.371	-1.695, -0.242	.01
	Medium	1.28	0.371	0.552, 2.005	< .001				
Mean corpuscular Hb (pg)	Small	0.91	0.153	0.679, 1.148	< .001	-0.408	0.119	-0.643, -0.174	< .001
	Medium	0.51	0.182	0.271, 0.740	< .001				
Mean corpuscular Hb concentration (g/L)	Small	3.48	0.690	2.130, 4.835	< .001	-1.930	0.690	-3.283, -0.578	< .001
	Medium	1.55	0.690	0.200, 2.905	.02				
Total iron binding capacity (umol/L)	Small	-14.07	1.375	-16.763, -11.374	< .001	8.764	1.375	6.068, 11.459	< .001
	Medium	-5.31	1.375	-8.000, -2.609	< .001				
Red blood cells (10 <sup>12</sup> /L)	Small	-0.09	0.050	-0.183, 0.012	.09	0.082	0.050	-0.016, 0.179	.10
	Medium	-0.00	0.50	-0.101, 0.094	.94				

\* Mixed linear regression models using Stata 12 Intercooled XP for Windows (College Station, Texas). For all eight models, parity category of the dam, age at weaning, and weaning-weight category were modelled as fixed effects and farm was modelled as a random effect (coefficient not shown), with iron status indicator as the dependent variable.

† Weight categories include small ( $5.2 \pm 1.5$  kg), medium ( $6.5 \pm 1.4$  kg), and large ( $7.5 \pm 1.6$  kg) (referent) piglets selected.

‡ Coefficient represents the change in each iron analyte 1 to 2 days prior to weaning, comparing small and medium-sized piglets to large piglets, eg, smaller piglets 1 to 2 days prior to weaning have hemoglobin values 3.4 g/L higher than larger piglets 1 to 2 days prior to weaning.

SE = standard error; CI = confidence interval; Hb = hemoglobin.

of iron by the piglet. Nevertheless, if iron is mixed with other products there is a risk that one of the products settles and when the compounded product is drawn into a syringe the proportion of iron may not be the expected concentration, so that some piglets are underdosed while others receive a high dose.

The rapid growth rate of modern piglets is a concern because iron requirements are likely increased. The large-sized piglets in this study had lower Hb concentrations at weaning than did the small and medium-sized piglets. Jolliff and Mahan<sup>14</sup> also found that heavier piglet weaning weight was associated with lower Hb and HCT values. The reason for this may be explained by the fact that each piglet receives a fixed amount of iron

from maternal stores.<sup>6</sup> Smaller piglets will have less blood volume, thus having a higher concentration of Hb for optimum synthesis. Larger piglets have a larger blood volume, therefore diluting Hb and increasing their iron requirements, making them more susceptible to iron deficiency and anemia. In this study, the larger pigs selected at weaning had lower Hb values than did the small and medium-sized pigs, indicating that a single 200-mg IM injection of either iron dextran or gleptoferron is not sufficient to prevent iron deficiency and anemia in some rapidly growing pigs.

The timing of the iron injection, specifically the age of the pig when iron is administered, is also important to consider when assessing Hb status at weaning. The producer from

each participating farm completed a questionnaire and indicated the age at which iron was administered. However, in reality, there was likely minor variation. It is unknown how stringently the producers followed their own iron-supplementation protocol, since it may not always be possible to administer iron on the same day of age after every litter of piglets is born. This limitation of the study may have introduced some misclassification bias to the variable “day of iron administration.” The literature indicates that parenteral iron supplementation within the first 3 to 4 days of age generally prevents anemia in suckling pigs.<sup>6</sup> In this study, the range of ages at which suckling pigs were administered iron was within the first 24 hours up until 7 days of age, and the majority of producers reported that they administered iron within 3 to 4 days of age.

**Table 3:** The mean ( $\pm$  SD) of various iron analytes in 1095 piglets sampled prior to weaning from 20 Ontario (Canada) commercial swine farms\*

Analyte	Small pigs (n = 365)	Medium pigs (n = 365)	Large pigs (n = 365)	All pigs
Hb (g/L)	114.1 ( $\pm$ 15.34)	113.3 ( $\pm$ 15.75)	110.6 ( $\pm$ 15.38)	112.7 ( $\pm$ 15.55)
Serum iron ( $\mu$ mol/L)	22.9 ( $\pm$ 11.73)	19.2 ( $\pm$ 12.94)	15.8 ( $\pm$ 11.80)	19.3 ( $\pm$ 12.50)
Hematocrit (L/L)	0.39 ( $\pm$ 0.05)	0.39 ( $\pm$ 0.05)	0.39 ( $\pm$ 0.05)	0.38 ( $\pm$ 0.50)
Mean corpuscular volume (fL)	66.7 ( $\pm$ 6.89)	65.6 ( $\pm$ 7.08)	64.4 ( $\pm$ 7.03)	65.6 ( $\pm$ 7.06)
Mean corpuscular Hb (pg)	19.8 ( $\pm$ 2.15)	19.3 ( $\pm$ 2.25)	18.8 ( $\pm$ 2.26)	19.3 ( $\pm$ 2.25)
Mean corpuscular Hb conc (g/L)	295.8 ( $\pm$ 12.46)	293.9 ( $\pm$ 11.35)	292.3 ( $\pm$ 12.44)	294.0 ( $\pm$ 12.17)
Total iron binding capacity ( $\mu$ mol/L)	74.4 ( $\pm$ 24.89)	83.3 ( $\pm$ 22.53)	88.5 ( $\pm$ 22.08)	82.1 ( $\pm$ 23.91)
Red blood cells ( $10^{12}$ /L)	5.8 ( $\pm$ 0.78)	5.9 ( $\pm$ 0.80)	5.9 ( $\pm$ 0.74)	5.9 ( $\pm$ 0.77)

\* Mean age at weaning,  $21.8 \pm 4.2$  days. Mean weights ( $\pm$  SD) at weaning: small pigs,  $5.2 \pm 1.5$  kg; medium pigs,  $6.5 \pm 1.4$  kg; large pigs,  $7.5 \pm 1.6$  kg.  
Hb = hemoglobin; SD = standard deviation; conc = concentration.

**Table 4:** The final model\* illustrating the effect of iron deficiency, anemia, weight and age at weaning, and parity at weaning on weight 3 weeks post weaning in pigs from 20 Ontario (Canada) commercial swine farms.

Variable	Coefficient	SE	95% CI	P
Hemoglobin status				
Iron deficient	-0.13	0.129	-0.385, 0.120	.30
Anemic	-0.82	0.259	-1.327, -0.313	< .01
Weight at weaning	1.25	0.037	1.177, 1.323	< .001
Parity				
2-5	0.34	0.157	0.035, 0.651	.03
> 5	0.50	0.187	0.137, 0.869	< .01
Age at weaning	0.12	0.023	0.072, 0.163	< .001

\* Mixed linear regression with farm modeled as random effect. Coefficients represent the change in weight (kg) 3 weeks post weaning if the variable is increased by one unit or compared to its referent category, eg, in piglets that were anemic (defined in Table 1) 1-2 days prior to weaning, 3-weeks-post-weaning weight was 0.82 kg lower than that in piglets with normal Hb. When a statistical contrast was conducted, anemic pigs at weaning had a 0.69-kg lower 3-weeks-post-weaning weight on average than that of pigs classified at weaning as iron deficient (defined in Table 1) ( $P < .05$ ). Referent parity was parity 1 (gilts). Mean age at weaning was  $21.8 \pm 4.2$  days.  
Hb = hemoglobin; SE = standard error; CI = confidence interval.

This may explain why this study did not find an association with timing of administration of iron supplementation, since the majority of farms administered iron products by the time pigs were 7 days of age.

The type of iron administered and the dose are important considerations when assessing Hb at weaning. Among the 20 participating farms, all farms supplemented their piglets with an IM injection of either a gleptoferron or iron dextran product. The majority of farms in this study used iron dextran. However, no difference was found in the Hb status of pigs when farms using iron dextran

and gleptoferron were compared. This finding is consistent with other studies that have reported no difference between gleptoferron and iron dextran in preventing iron deficiency and anemia.<sup>26,27</sup> This suggests that iron from both gleptoferron and iron dextran is utilized with comparable efficacy for hemoglobin synthesis and iron storage in young growing pigs. On most farms, pigs have access to creep feed containing iron. It was not possible in the current study to determine whether the intake of creep feed contributed to the piglet's iron status. It was thought that because most piglets were weaned at approxi-

mately 3 weeks of age, only a small amount of feed would have been consumed and may not have been a factor in meeting the piglet's iron needs.

A different test method was used to assess Hb status at 3 weeks post weaning than was used to assess the suckling piglets. Testing method is confounded with differences between Hb at weaning and 3 weeks post weaning. Hemoglobin status has been evaluated using a handheld device on farm.<sup>28-30</sup> During the second visit to each participating farm, Hb measurements were evaluated using a STAT-Site M Hgb handheld meter.



**Table 5:** Model\* assessing the association of zinc oxide concentration in feed and odds of anemia in piglets 3 weeks post weaning 20 Ontario (Canada) commercial swine farms.

Variable	OR	SE	95% CI	P
Zinc oxide concentration				
High dose	3.4	1.974	1.114-10.595	.03
Very high dose	4.1	2.551	1.206-13.889	.02
Hemoglobin at weaning	1.0	0.006	0.971-0.996	< .01
Weight at weaning	0.9	0.052	0.822-1.025	.13
Iron administered	2.9	1.454	1.086-7.749	.03

\* Mixed logistic regression. OR represents the odds of anemia, eg, the odds of nursery pigs being anemic was 3.4 times greater for pigs consuming high doses of zinc oxide in feed than for those consuming a nutritional dose of zinc oxide in feed. The referent was the nutritional dose ( $\leq 500$  mg/kg of feed), the high dose was 2000-3000 mg/kg of feed, and the very high dose was  $> 3000$  mg/kg of feed). The referent for the type of iron administered was gleptoferron.  
OR = odds ratio; SE = standard error; CI = confidence interval.

This convenient handheld meter can be utilized while on farm to assess analytical results more rapidly than by submitting samples to a laboratory service. A limitation to using this particular handheld device is that it has not been used to assess Hb concentration in swine. However, this device has been tested on humans and has a 0.93 correlation coefficient when compared to standard laboratory testing.<sup>31</sup> Another handheld meter, the HemoCue, is a similar device that has been used to assess Hb measurements in both humans and swine.<sup>28,29</sup> This device measures Hb content via the conjugation of free Hb to azidemethemoglobin, photometrically measured at 570 nm, whereas the STAT-Site M Hgb meter uses the same method but measures photometrically at 565 nm.<sup>29,30</sup> Kutter et al<sup>28</sup> reported good agreement after assessing the HemoCue meter with standardized Hb laboratory measurements. The sensitivity and specificity of the HemoCue meter were 97% and 100%, respectively.<sup>31</sup>

Similar to suckling piglets, nursery pigs also grow rapidly, resulting in a rapid increase in blood volume and a high nutritional iron requirement.<sup>32</sup> The decrease in Hb concentrations when the nursery pigs were tested using the STAT-Site M Hgb meter suggests that Hb synthesis may not increase proportionally as the rapidly growing nursery pigs increase in weight and blood volume.<sup>14</sup> There are several possible reasons why iron deficiency and anemia continued 3 weeks post weaning. Firstly, the types of iron used in the nursery diets may have varied among farms, some being more readily absorbed than others. Although the iron concentrations varied in the nursery diets, all were well over

National Research Council requirements. The stress of weaning is also associated with a reduction in feed consumption, which may play a role in iron deficiency and anemia post weaning.<sup>33</sup> In a previous study, it was suggested that intestinal regulation of iron absorption might not be entirely functional within the first few weeks post weaning.<sup>34</sup> Divalent metal transporter 1 (DMT1) is an important membrane protein that plays a key role in intestinal iron absorption as well as iron transport.<sup>35</sup> Hansen et al<sup>32</sup> found that mRNA transcript levels of DMT1 are not up-regulated in pigs until they reach 26 to 27 days of age. Therefore, there is evidence that iron absorption and transport are not regulated in pigs until they are in the nursery. In addition, iron absorption is controlled by hepcidin antimicrobial peptide (HAMP), which is derived from the liver and is produced in response to high concentrations of iron in the feed.<sup>34</sup> HAMP binds to ferroportin (an iron exporter) on cell surfaces and degrades the cells.<sup>36</sup> Hansen et al<sup>32</sup> also concluded that HAMP-mediated iron homeostasis is likely not fully functional in newly weaned pigs and that these pigs are not able to properly react to changes in dietary iron. In the current study, piglets that were anemic grew more slowly. This might lead to economic issues in the future if mortality and morbidity rates are elevated due to underlying anemia.

Prevention of iron deficiency in piglets at the time of weaning needs to be investigated. There is a danger of iron toxicosis and a concern of increasing bacteremia if the dosage of iron administered within the first few days

of life is higher than that recommended by the manufacturer. A second injection close to or at the time of weaning may be a good way to provide sufficient iron to the pig to meet the nutritional demands of the early weaning stage without increasing the risk of toxicity, but a second injection is associated with an increased labor cost. There may be an economic benefit to adding a second injection to the overall benefit of piglet health and performance that might outweigh the cost of labor, but this needs to be assessed on individual farms and warrants further study. However, Peters and Mahan<sup>37</sup> found that suckling pigs that were injected with 200 mg of iron at birth and then a second time at weaning did not respond to the additional injection, and thus this also needs to be further investigated.

Iron deficiency and anemia was associated with lower growth rates (poor nursery performance) in this study. Pigs that were iron deficient and anemic at weaning had mean 3-week nursery body weights lower than pigs with normal Hb levels at weaning, and this is consistent with other reports.<sup>38,39</sup> Schrama et al<sup>38</sup> found that piglets with low Hb values had lower ADG than piglets with higher Hb levels. Gentry et al<sup>39</sup> also found that pigs with higher Hb levels at weaning had greater ADG and higher feed intake post weaning. These results are comparable to the current study, since 3-weeks-post-weaning weight was positively associated with Hb status at weaning.

In order to investigate the effects of Hb status on post-weaning performance, various farm management protocols were accounted for in the analyses. Piglet weaning weight and age were controlled for because they are both

significant contributors to 3-week growth performance in nursery pigs. Piglet weight at weaning was positively associated with 3-weeks-post-weaning weight in the nursery barn, which is consistent with previous studies.<sup>40,41</sup> Piglets weaned at an older age reached a greater 3-weeks-post-weaning weight than did piglets weaned at an earlier age.

Both litter size and sex were not included in the final model because these variables had no significant association with 3-weeks-post-weaning weight in univariable analysis. Sow parity was included in the final model, as there was a statistically significant association with 3-weeks-post-weaning weight in univariable analysis, as well as in the final model. In a previous study conducted by Smith et al,<sup>42</sup> piglets born to primiparous sows had a slower growth rate than piglets from sows with a higher parity. The same was found in the current study, as piglets from higher parity sows had higher 3-weeks-post-weaning body weights than piglets from primiparous sows.

High concentrations of in-feed zinc oxide ( $\geq 2000$  mg per kg) are commonly used therapeutically in starter diets to control post-weaning *Escherichia coli* diarrhea.<sup>43-45</sup> A possible reason why nursery pigs had greater odds of being anemic when consuming high and very high doses of in-feed zinc oxide, compared to a nutritional dose of zinc oxide, is that high doses of zinc interfere with conversion of iron into ferritin.<sup>43</sup> It is also possible that both high and very high levels of zinc in feed may increase the iron requirement in young growing pigs, because zinc decreases the life span of the red blood cell.<sup>43</sup> Zinc, copper, and iron are metals that interact and may present competitive inhibition of transport and bioavailability.<sup>46,47</sup> Therefore, the individual interactions between metals such as copper and zinc may affect iron absorption. In aqueous solutions and at higher doses, competition between metals with similar properties can occur.<sup>48</sup> There are many inhibitory interactions among these metals that could occur when high doses of a certain metal are given.<sup>48</sup> In a competition study, when the concentrations of copper and zinc were increased, iron uptake decreased.<sup>48</sup> In other studies,<sup>49,50</sup> zinc status influenced iron uptake, indicating that DMT1 may not simultaneously transport iron and zinc. A limitation of the current study is that the length of time during which the pigs were fed the nursery diets with the specific concentration of zinc oxide were not measured.

The use of high concentrations of in-feed zinc is likely to interfere with iron absorption, and thus should be examined further in future studies.

Copper is another mineral that could have an effect on iron deficiency and anemia in young growing pigs. However, due to the lack of variability of in-feed copper content among the participating farms in this study, copper could not be controlled for. Both copper and zinc are heavy metals that are used therapeutically in feed. Although some herds used nutritional doses of zinc oxide ( $\leq 500$  mg per kg of feed), all herds used high levels of copper sulphate (125 mg per kg of feed).

The economic impact of inadequate iron supplementation in piglets is unknown; however, iron status can be easily evaluated and corrected at minimal additional cost. Moreover, any impact iron deficiency may have on growth rates could negatively affect the cost of production. Therefore, evaluation and correction of iron deficiency or anemia would outweigh the minimal added cost associated with iron supplementation, since this will improve weight gain and overall welfare of the piglets.

In summary, this study identified iron deficiency and anemia in newly weaned pigs and in pigs 3 weeks post weaning. There was evidence that anemia is associated with a negative impact on post-weaning growth performance. The widespread prevalence of iron deficiency and anemia on almost all farms in this study indicates that iron status should be monitored on all farms and supplementation programs assessed.

## Implications

- Iron supplementation protocols used by these participating farms were not sufficient to meet iron requirements of large, fast-growing suckling pigs.
- Iron deficiency and anemia may persist beyond 3 weeks in the nursery.
- Anemia is negatively associated with post-weaning growth.
- Under the conditions of this study, high dietary concentrations of zinc oxide ( $> 2000$  mg per kg of feed) are associated with a higher risk of anemia in weaned pigs

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## Conflict of interest

None reported

## Disclaimer

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## References

1. Kernkamp HCH, Clawson AJ, Ferneyhough RH. Preventing iron-deficiency anemia in baby pigs. *J Anim Sci.* 1962;21:527–532.
2. Dale DG, MacDonald MA, Moxley JE. Hemoglobin levels of piglets at birth and at 21 days and their relation to weight at 154 days of age. *Can J Comp Med Vet Sci.* 1961;25:193–197.
3. Venn JAJ, McCance RA, Widdowson EM. Iron metabolism in piglet anaemia. *J Comp Pathol Therap.* 1947;57:314–325.
- \*4. Miller ER, Ullrey DE. *Baby pig anemia*. Cooperative Extension Service, College of Agriculture. Michigan State University. 1977. Available at: <http://old.pork.org/filelibrary/factsheets/pigfactsheets/newfactsheets/04-01-07g.pdf>. Accessed 9 July 2015.
5. Murphy KA, Friendship RM, Dewey CE. Effects of weaning age and dosage of supplemented iron on the hemoglobin concentrations and growth rate of piglets. *Swine Health Prod.* 1997;5:135–138.
6. Calvo JJ, Allue JR. Plasma ferritin and other parameters related to iron metabolism in piglets. *Comp Biochem Phys A.* 1986;85:471–476.
7. Lipiński P, Starzyński RR, Canonne-Hergaux F, Tudek B, Oliński R, Kowalczyk P, Dziaman T, Thibaudeau O, Gralak M, Smuda E, Woliński J, Usińska A, Zabielski R. Benefits and risks of iron supplementation in anemic neonatal pigs. *Am J Pathol.* 2010;177:1233–1243.
8. Starzyński RR, Laarakkers CM, Tjalsma H, Swinkels DW, Pieszka M, Styś A, Mickiewicz M, Lipiński P. Iron supplementation in suckling piglets: How to correct iron deficiency anemia without affecting plasma hepcidin levels. *PLoS one.* 2013;8(5):e64022.
9. Svoboda M, Drabek J. Iron deficiency in suckling piglets: etiology, clinical aspects and diagnosis. *Folia Vet.* 2005;49:104–111.
10. Wei KQ, Xu ZR, Luo XG, Zeng LL, Chen WR, Timothy MF. Effects of iron from an amino acid complex on the iron status of neonatal and suckling piglets. *Am J Anim Sci.* 2005;18:1485–1491.
- \*11. Nielsen JP, Busch ME, Friendship R, Martineau G-P, Framstad T. Herd diagnosis of iron deficiency in piglets. *Proc Eur Symp Porc Health Management.* Edinburgh, UK. 2013:168.

12. Bhattarai S, Nielsen JP. Early indicators of iron deficiency in large piglets at weaning. *J Swine Health Prod.* 2015;23:10–17.
13. Bermejo F, García-López, S. A guide to diagnosis of iron deficiency and iron deficiency anemia in digestive diseases. *World J Gastroenterol.* 2009;15:4638–4643.
14. Jolliff JS, Mahan DC. Effect of injected and dietary iron in young pigs on blood hematology and postnatal pig growth performance. *J Anim Sci.* 2011;99:4068–4080.
15. Egeli AK, Framstad T. An evaluation of iron-dextran supplementation in piglets administered by injection on the first, third or fourth day after birth. *Res Vet Sci.* 1999;66:179–184.
16. Rinker MJ, Hill GM, Link JE, Meyer AM, Rowntree JE. Effects of dietary zinc and iron supplementation on mineral excretion, body composition, and mineral status of nursery pigs. *J Anim Sci.* 2005;83:2762–2774.
17. Cobas Iron Gen.2 [package insert]. Indianapolis, Indiana: Roche Diagnostics; 2012.
18. Vanzetti G. An azide-methemoglobin method for hemoglobin determination in blood. *J Lab Clin Med.* 1966;67:116–126.
- \*19. Government of Canada. Feeds Regulations, 1983 (SOR per 83-593). 2009. Available at: [http://per.laws-lois.justice.gc.ca/per/eng/per/regulations/per\\_sor-83-593/per\\_FullText.html](http://per.laws-lois.justice.gc.ca/per/eng/per/regulations/per_sor-83-593/per_FullText.html). Accessed 24 September 2015.
- \*20. Miller ER. Vitamins and minerals for nursing pigs: Iron. *Proc AASP.* Des Moines, Iowa. 1977:2.
21. Dukes HH, Swenson MJ. *Dukes' Physiology of Domestic Animals.* 8th ed. Ithaca, New York: Comstock Publishing Associates. 1970:310.
22. Cook JD. Diagnosis and management of iron-deficiency anemia. *Best Pract Res Clin Haematol.* 2005;18:319–332.
- \*23. Walsh S, Friendship R, Amezcua R, Nielsen J, Busch M, Martineau G, Framstad T. Effects of an iron dextran injection at weaning on pigs' blood hemoglobin status and nursery performance. *Proc AASV.* Dallas, Texas. 2014:313.
24. CQA® Producer Manual Version 2.1 [D4 Medical Supplies: Use and Storage]. Ottawa, Canada: Canadian Pork Council; 2007. Available at: <http://www.cqa-aqc.com/documents/producer-manual/CurentVersions2013.pdf>. Accessed 14 July 2015.
25. Policy on Manufacturing and Compounding Drug Products in Canada POL-0051. Health Canada per Health Products and Food Branch Inspectorate. Policy on Manufacturing and Compounding Drug Products in Canada. Ottawa, Ontario, Canada; 2009. Available at: [http://www.hc-sc.gc.ca/dhp-mps/alt\\_formats/hpfb-dgpsa/pdf/compli-conform/pol\\_0051-eng.pdf](http://www.hc-sc.gc.ca/dhp-mps/alt_formats/hpfb-dgpsa/pdf/compli-conform/pol_0051-eng.pdf). Accessed 9 July 2015.
26. Pollmann DS, Smith JE, Stevenson JS, Schoneweis DA, Hines RH. Comparison of gleptosil with iron dextran for anemia prevention in young pigs. *J Anim Sci.* 1983;56:640–644.
27. Smith JE, Moore K, Boyington D, Pollmann DS, Schoneweis D. Serum ferritin and total iron-binding capacity to estimate iron storage in pigs. *Vet Pathol.* 1984;21:597–600.
28. Kutter A, Mauch J, Riend B, Martin-Jurado O, Spielmann N, Weiss M, Bettschart-Wolfensberger R. Evaluation of two devices for point-of-care testing of haemoglobin in neonatal pigs. *Lab Anim.* 2012;46:65–70.
29. Callan M, Giger U, Oakley D, Scotti M, Shofer F. Evaluation of the hemocue system for hemoglobin measurement in animals. *J Vet Intern Med.* 1992;6:141.
30. Rincker MJ, Hill GM, Link JE, Rowntree JE. Effects of dietary iron supplementation on growth performance, hematological status, and whole-body mineral concentrations of nursery pigs. *J Anim Sci.* 2004;82:3189–3197.
31. STAT Site M<sup>Hgb</sup> Whole Blood Hemoglobin Test Cards [package insert]. Boerne, Texas: Stanbio Laboratory; 2012.
32. Hansen SL, Trakooljul N, Spears JW, Liu HC. Age and dietary iron affect expression of genes involved in iron acquisition and homeostasis in young pigs. *J Nutr.* 2010;140:271–277.
33. Pluske JR, Hampson DJ, Williams IH. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest Prod Sci.* 1997;51:215–236.
34. Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science.* 2004;306:2090–2093.
35. Priwitzzerova M, Nie G, Sheftel AD, Pospisilova D, Divoky V, Ponka P. Functional consequences of the human DMT1 (SLC11A2) mutation on protein expression and iron uptake. *Blood.* 2005;106:3985–3987.
36. Svoboda M, Drabek J. Iron deficiency in suckling piglets: etiology, clinical aspects and diagnosis. *Folia Vet.* 2004;49:104–111.
37. Peters JC, Mahan DC. Effects of neonatal iron status, iron injections at birth, and weaning in young pigs from sows fed either organic or inorganic trace minerals. *J Anim Sci.* 2008;86:2261–2269.
38. Schrama JW, Schouten JM, Swinkels JW, Gentry JL, de Vries Reilingh G, Parmentier HK. Effect of hemoglobin status on humoral immune response of weanling pigs differing in coping styles. *J Anim Sci.* 1997;75:2588–2596.
39. Gentry JL, Swinkels JW, Lindemann MD, Schrama JW. Effect of hemoglobin and immunization status on energy metabolism of weanling pigs. *J Anim Sci.* 1997;75:1032–1040.
40. de Grau A, Dewey C, Friendship R, de Lange K. Observational study of factors associated with nursery pig performance. *Can Vet J.* 2005;69:241–245.
41. Wolter BF, Ellis M. The effects of weaning weight and rate of growth immediately after weaning on subsequent pig growth performance and carcass characteristics. *Can J Anim Sci.* 2001;81:363–369.
42. Smith AL, Stalder KJ, Serenius TV, Baas TJ, Mabry JW. Effect of piglet birth weight on weights at weaning and 42 days post weaning. *J Swine Health Prod.* 2007;15:213–218.
43. Henry PR, Miller ER. Iron bioavailability. In: Ammerman CB, Baker DP, Lewis AJ. *Bioavailability of Nutrients for Animals.* San Diego, California: Academic Press; 1995:175.
44. Amezcua R, Friendship R, Dewey CE. An investigation of the presence of *Escherichia coli* O149:K91:F4 on pig farms in southern Ontario and the use of antimicrobials and risk factors associated with the presence of this serogroup. *Can Vet J.* 2008;49:39–45.
45. Slifierz MJ, Friendship R, Weese JS. Zinc oxide therapy increases prevalence and persistence of methicillin-resistant *Staphylococcus aureus* in pigs: A randomized controlled trial. *Zoonoses Public Health.* 2015;62:301–308.
46. Reinstein NH, Lonnerdal B, Keen CL, Hurler LS. Zinc-copper interactions in the pregnant rat: Fetal outcome and maternal and fetal zinc, copper and iron. *J Nutr.* 1984;114:1266–1279.
47. Brewer GJ, Hill GM, Dick RD, Prasad AS, Cossack ZT. Interaction of trace elements: clinical significance. *J Am Coll Nutr.* 1985;4:33–38.
48. Arredondo M, Martinez R, Nunez MT, Ruz M, Olivares M. Inhibition of iron and copper uptake by iron, copper and zinc. *Biol Res.* 2006;39:95–102.
49. Iyengar V, Pullakhandam R, Nair KM. Iron-zinc interaction during uptake in human intestinal Caco-2 cell line: kinetic analyses and possible mechanism. *Indian J Biochem Biophys.* 2009;46:299–306.
50. Espinoza A, Le Blanc S, Olivares M, Pizarro F, Ruz M, Arredondo M. Iron, copper, and zinc transport: inhibition of divalent metal transporter 1 (DMT1) and human copper transporter 1 (hCTR1) by shRNA. *Biol Trace Elem Res.* 2012;146:281–286.

\* Non-refereed references.

