Early indicators of iron deficiency in large piglets at weaning

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Summary

Objective: To investigate whether large piglets at weaning have indications of iron deficiency anemia.

Materials and methods: The study was carried out in five conventional high-performing farrow-finish Danish sow herds. Within each herd, litters belonging to a weekly farrowing batch close to weaning were identified, and 20 litters were randomly selected. From each litter the largest piglet (Large), a random piglet (Random), and the smallest piglet (Small) were chosen. Blood samples collected at weaning from the selected piglets were subjected to hematological analysis, including serum iron and total iron-binding capacity (TIBC).

Results: A total of 296 piglets belonging to 100 litters were included in the study. The blood hemoglobin concentrations in Large, Random, and Small piglets were 119.6 ± 15.5, 121.5 \pm 15.0, and 121.5 \pm 13.2 g per L, respectively, which did not differ significantly. However, large piglets had significantly lower mean corpuscular hemoglobin, reticulocyte cellular volume, reticulocyte hemoglobin content, mean reticulocyte corpuscular hemoglobin concentration, serum iron, and transferrin saturation than did Random and Small piglets. In accordance with this, Large piglets had significantly higher red blood cell distribution width, reticulocyte red cell distribution width, and TIBC than did Random and Small piglets.

Implications: Large piglets in a litter are at a higher risk of developing iron deficiency anemia at weaning than are smaller piglets. Alternative hematological indices might serve as better early indicators of iron deficiency rather than traditionally used hemoglobin values.

Keywords: swine, size, hematology, weaning, iron deficiency anemia

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Resumen - Indicadores tempranos de deficiencia de hierro en lechones grandes al destete

Objetivo: Investigar si los lechones grandes al destete tienen indicios de anemia por deficiencia de hierro.

Materiales y métodos: El estudio se llevó a cabo en cinco hatos porcinos Daneses convencionales de parto a finalización de alto desempeño. Dentro de cada hato, se identificaron camadas pertenecientes a un grupo de hembras de partos semanales cercanas al destete, y se seleccionaron 20 camadas al azar. Dentro de cada camada, se seleccionaron el lechón más grande (Grande), un lechón al azar (Azar), y el lechón más pequeño (Pequeño). Las muestras de sangre recolectadas al destete de los lechones seleccionados se sometieron a análisis hematológicos, incluyendo hierro en el suero y la

capacidad total de adición de hierro (TIBC por sus siglas en inglés).

Resultados: En el estudio, se incluyeron un total de 296 lechones pertenecientes a 100 camadas. Las concentraciones de hemoglobina de la sangre en los lechones Grande, Azar, y Pequeño fueron 119.6 ± 15.5, 121.5 \pm 15.0, y 121.5 \pm 13.2 g por L, respectivamente, lo cual no difirió significativamente. Sin embargo, los cerdos grandes tuvieron significativamente menos hemoglobina corpuscular media, volumen celular de reticulocitos, contenido de hemoglobina reticulocitaria, concentración media de hemoglobina corpuscular reticulocitaria, hierro en suero, y saturación de transferrina que los lechones Azar y Pequeño. De acuerdo con esto, los cerdos grandes tuvieron, significativamente, un ancho de distribución de glóbulos rojos, ancho de distribución de

glóbulos rojos reticulocitarios, y TIBC más altos que los lechones Azar y Pequeño.

Implicaciones: Los lechones grandes en una camada corren un mayor riesgo de desarrollar anemia por deficiencia de hierro al destete que los lechones más pequeños. Además, los índices hematológicos alternativos pueden servir como mejores indicadores tempranos de deficiencia de hierro que los valores de hemoglobina utilizados tradicionalmente.

Résumé - Indicateurs précoces de déficience en fer chez des gros porcelets au sevrage

Objectif: Déterminer si au moment du sevrage les gros porcelets présentent des indications d'anémie par déficience en fer.

Matériels et méthodes: Cette étude a été réalisée dans cinq troupeaux conventionnels de type naisseur-finisseur sur des truies Danoises de haute performance. Dans chaque troupeau, les portées appartenant à un lot hebdomadaire de mise-bas près du moment du sevrage furent identifiées, et 20 portées furent choisies au hasard. De chaque portée le plus gros porcelet (Gros), un porcelet pris au hasard (Hasard), et le plus petit porcelet (Petit) furent choisis. Des échantillons sanguins prélevés au moment

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du sevrage à partir des porcelets sélectionnés furent soumis à une analyse hématologique, incluant une mesure du fer sérique et de la capacité totale de liaison du fer (TIBC).

Résultats: Un total de 296 porcelets provenant de 100 portées furent inclus dans l'étude. Les concentrations d'hémoglobine sanguine chez les porcelets Gros, au Hasard, et Petit étaient de 119 ± 15,5, 121,5 ± 15,0, et 121.5 ± 13.2 g par L, respectivement, et ne différaient pas de manière significative. Toutefois, les porcelets Gros avaient des valeurs significativement moindre que les porcelets au Hasard et les Petits porcelets de la quantité d'hémoglobine corpusculaire moyenne, du volume cellulaire et du contenu en hémoglobine des réticulocytes, de la concentration moyenne en hémoglobine des réticulocytes corpusculaires, du fer sérique, et de la saturation de la transferrine. En accord avec ces observations, les porcelets Gros avaient une distribution significativement plus étendue des érythrocytes et des réticulocytes des globules rouges, ainsi que de la TIBC comparativement au porcelets au Hasard et les Petits porcelets.

Implications: Les porcelets gros dans une portée ont un risque plus élevé de développer une anémie par déficience en fer au sevrage que les porcelets plus petits. De plus, des indices hématologiques alternatifs pourraient servir de meilleurs indicateurs de déficience en fer comparativement aux valeurs en hémoglobine utilisées de manière plus traditionnelle.

piglets are born with very limited iron reserves. Iron content in the piglet at birth covers the requirement for only the first 3 to 4 days. Furthermore, sow milk contains very little iron. In intensive housing systems without access to soil, iron supplementation is necessary to prevent iron deficiency anemia and allow a high growth rate. Therefore, injection of iron in the first days of life has become a routine management practice in commercial Danish herds for prevention of anemia and iron deficiency.

Additional iron may be available for piglets during the lactation period in standard creep feed or in special oral iron formulations. However, iron uptake via the oral route may be inconsistent and of limited quantity if sufficient sow milk is available. Furthermore, the calcium content of milk interferes with intestinal iron absorption.²

There is a large variation in the birth weight of piglets born to large litters.^{3,4} Within a litter, piglets larger at birth tend to grow faster than the smaller ones because of their capability to compete for sow's milk.³ However, iron dosing regimens are based on a standard dose of 200 mg iron irrespective of the birth weight, growth rate, or weaning age of the piglets.

Previously,⁵ it has been demonstrated that hemoglobin (Hb) and hematocrit (Hct) were significantly lower by 17 days of age in heavier and fast-growing piglets than in lighter piglets that were injected with 200 mg iron at birth. This suggests that the iron stores after injection in the first days of life are depleted around weaning, the critical time for iron deficiency and anemia to develop in piglets.

Hemoglobin concentration measurement has been the most widely used method to ensure optimum iron status in piglets. However, Hb measurement alone is not sensitive enough to detect an early fall in iron status.^{6,7} Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), serum iron, total iron-binding capacity (TIBC), transferrin saturation (TfS), serum ferritin, and indices of reticulocytes are some of the commonly reported early indicators of iron deficiency described in the literature in both human and pig studies.⁷⁻¹⁵ Therefore, our study aimed to investigate whether large piglets at weaning had indications of iron deficiency anemia, as determined by Hb and other hematological and hematochemical values.

Materials and methods

The present study was not subject to ethical approval, as Danish laws do not require ethical approval for studies involving only standard diagnostic procedures of direct relevance to herd-health management.

Herd selection

Five conventional, high-performing, farrow-finish sow herds were recruited by courtesy of two large specialized pig practices in Denmark (Table 1). The selection criteria used were herd size of approximately 1000 sows, with farrowing batches of at least 45 sows and herds providing a single injection of iron, administered either intramuscularly (IM) or subcutaneously (SC) during the first few days of life. All selected herds had high health status and had no obvious health challenges at the time of the study. The herds followed similar strategies (single injection of iron) to prevent iron deficiency and

anemia in piglets, which is the most common practice in Danish herds. Herds using oral iron supplementation, those selling the weaners to other herds, and breeding herds were excluded. The study was conducted between July and September 2013.

Piglet selection

Within each herd, all litters belonging to a weekly farrowing batch that were as close as possible to weaning were identified, and 20 litters were randomly selected. Then, within each litter, three piglets were selected. First, a random piglet (Random) was selected systematically: the observer stood in the same position in front of each pen and counted snouts from the front of the pen, then chose the sixth piglet counted. This systematic random sampling procedure was repeated for each litter. The smallest piglet (Small) and the largest piglet (Large) were purposively chosen by visually judging their sizes. Piglets were selected by the same observer each time to avoid bias. Litters receiving extra iron supplementation, litters from a nurse sow, and piglets suffering from obvious severe unthriftiness or disease were excluded from the study. A nurse sow was defined as a sow receiving piglets born in weekly batches other than her own. Feeding and management practices were carried out by the farmer as per the routine standards of the particular farm. Piglets in these herds were injected with iron at 3 to 4 days of age, and all male piglets were castrated in the first few days of life.

Data collection and hematology

Within each litter, farrowing date of the sow was recorded and each selected piglet was weighed individually. After placement of each piglet in dorsal recumbency, approximately 3 mL of blood was withdrawn by puncture of the anterior vena cava into one plain and one EDTA evacuated blood vial.

The EDTA samples were stored at 4°C and analysed in the laboratory within 2 days of collection, while serum samples were frozen (-20°C) until analysis. The EDTA blood samples were analysed for hematological indices: erythrocyte count (RBC), total and differential leukocyte count, platelets, RDW, Hb, hemoglobin distribution width (HDW), Hct, MCV, MCH, and mean corpuscular Hb concentration (MCHC). Reticulocyte indices were also determined, which included reticulocyte count (absolute and relative), reticulocyte hemoglobin content (CHr), mean reticulocyte corpuscular Hb concentration (CHCMr), reticulocyte

Table 1: Descriptive data from five Danish swine herds participating in a study to investigate whether large piglets at weaning had indications of iron deficiency anemia*

	Herd 1	Herd 2	Herd 3	Herd 4	Herd 5
Weaning age (days)	26.2	24.1	27.8	24.9	25.7
Total born alive/litter	15.7	15.6	15.7	14.5	14.3
Herd size (sows)	1101	1100	1155	1201	940
Age at injection (days)	3-4	3-4	4	4	3
Iron brand name†	Solofer	Ursoferran	Ursoferran	Hyofer	Hyofer
Dose (route)	1 mL (IM)	1.1 mL (IM)	1 mL (IM)	1 mL (SC)	1.5 mL (IM)
Creep feed start (days of age)	5	7	10	6	14

- * Five farrow-finish sow herds were recruited from different regions of Denmark by courtesy of two specialized pig practices. All piglets were injected with an iron supplement during the first few days of life. Iron deficiency anemia was determined by hemoglobin and other hematological and hematochemical values in blood samples collected at weaning.
- † Each iron product contained 200 mg iron dextran/mL: Solofer (Pharmacosmos A/S, Holbæk, Denmark); Ursoferran (Serumwerk Bernburg AG, Bernburg, Germany); and Hyofer (Salfarm Danmark A/S, Kolding, Denmark).

IM = intramuscular; SC= subcutaneous.

cellular volume (MCVr), reticulocyte red cell distribution width (RDWr), and reticulocyte Hb distribution width (HDWr). The values of Hb obtained from the laboratory were converted from mmol per L to g per L by multiplying by 16.11. The serum samples were analysed for serum iron and TIBC. Transferrin saturation (TfS) was calculated using the formula

TfS (%) = (serum iron \div TIBC) × 100.

All hematological testing was performed using the Advia 2120i Hematology System (Siemens Healthcare Diagnostics Inc, Tarrytown, New York), while serum iron and TIBC were tested using the Advia 1800 Chemistry System (Siemens Healthcare Diagnostics Inc) at the Central Laboratory, Department of Veterinary Clinical and Animal Sciences, University of Copenhagen.

Statistical analysis

Data analysis was performed using SAS 9.3 (SAS Institute Inc, Cary, North Carolina). All data were presented as mean and standard deviation (SD). For normally distributed data, a one-way ANOVA was used to calculate the difference in parameters among the three sizes of piglets. Non-normal data were transformed using either square root or log transformation in order to obtain a normal distribution before analysis. For the variables that remained non-normal, a non-parametric test (the Kruskal-Wallis test) was used, and in case of significance (P < .05), pairwise comparisons were made using the Wilcoxon rank sum test. Herd and litter

effects were not considered because all piglet selection was matched on litters.

The Hb values were categorized into three groups using reference values (90 to 112 g per L)¹⁶ for piglets 20 days old: Low Hb (< 90 g per L), Moderate Hb (90 to 110 g per L) and High Hb (> 110 g per L). Piglets with Low Hb values were considered anemic. The differences in prevalence of anemia in the three categories among the three piglet sizes were determined using the Fisher exact test. Statistical significance was set at P < .05 for all tests.

Results

A total of 296 piglets belonging to 100 litters were included in the study. Four piglets were removed from the study before the blood samples were collected. Hemolyzed blood samples from 33 piglets were discarded, serum samples were missing from four piglets, and one erroneous Hb value was removed during statistical analysis. The average weaning age of the piglets was 25.7 ± 2.2 days (Table 2). Large piglets had significantly higher weaning weights than did Random (P < .001) and Small (P < .001) piglets. Similarly, Random piglets were heavier than Small ones (P < .001).

Hematological and hematochemical parameters

The mean Hb concentrations (\pm standard deviaton) in Large, Random, and Small piglets were 119.6 \pm 15.5, 121.5 \pm 15.0, and 121.5 \pm 13.2 g per L, respectively, which did not differ significantly (P = .75). The Hb concentrations of piglets of different sizes in five herds are presented in Table 3.

Large piglets had lower MCH, MCVr, CHr, CHCMr, serum iron, and TfS than did Random piglets (P = .02, P < .01, P < .001, P = .001, P = .001, and P < .001, respectively) and Small piglets (P = .03, P < .01, P < .001, P < .001, P < .001, and P < .001, and P < .001, respectively). Mean serum iron concentrations for the three piglet categories are shown in Figure 1. The concentrations of all measured parameters in piglets of different sizes are shown in Table 4, and normal hematological values reported in the literature for piglets are shown in Table 5.

Large piglets had higher RDW, RDWr, and TIBC than did Random piglets (P < .001, P < .01 and P < .01, respectively) and Small piglets (P = .02, P < .001. and P < .001, respectively). Total iron-binding capacity for piglets in the three categories is shown in Figure 2. The percentages of basophils, lymphocytes, and monocytes were higher (P = .001, P < .001, and P = .02, respectively), but percent neutrophils was lower (P < .001), in Large piglets than in Small piglets.

Table 2: Mean ages and weaning weights of 300 piglets selected for collection of blood samples for hematological assays*

A co (days)	Minimum	Maximum	Mean (SD)
Age (days)	21	33	25.7 (2.21)
Body weight (kg)			
Large piglet	5.1	11.7	7.95 (1.5) ^a
Random piglet	3.7	11.5	6.2 (1.38) ^b
Small piglet	2.5	7.0	4.57 (0.9) ^c

^{* 20} litters as close as possible to weaning were chosen from each of the five selected farrow-finish sow herds in Denmark (described in Table 1). From each litter, the visually largest piglet (Large), the visually smallest piglet (Small), and a random piglet (Random) were selected for data collection (n = 100 for each size category).

Table 3: Mean age, weight, and hemoglobin concentration at weaning in piglets selected for data collection from five Danish farrow-finish herds*

Piglet size	n†	Mean age (days) (SD)	Mean body weight (kg) (SD)	Mean Hb (g/L) (SD)
Large	19		9.3 (1.2)	114.8 (17.3)
Random	17	26.2 (0.6)	6.9 (1.2)	121.6 (10.7)
Small	19		4.6 (1.1)	124.7 (11.4)
Large	17		7.7 (1.5)	125.8 (8.9)
Random	16	24.1 (1.5)	5.8 (1.4)	128.5 (10.5)
Small	20		4.5 (0.9)	122.9 (12.1)
Large	14		7.7 (1.4)	112.6 (15.1)
Random	17	27.8 (3.1)	6.1 (1.2)	119.6 (12.6)
Small	18		4.9 (0.8)	119.0 (11.1)
Large	20		5.5 (1.1)	113.2 (15.5)
Random	18	24.9 (1.5)	5.4 (1.0)	111.7 (17.1)
Small	18		4.2 (0.7)	116.7 (19.0)
Large	15		8.5 (0.9)	133.7 (5.3)
Random	16	25.7 (0.7)	6.6 (1.1)	127.5 (17.6)
Small	18		4.5 (0.7)	123.6 (10.1)
	Large Random Small Large Random	Large 19 Random 17 Small 19 Large 17 Random 16 Small 20 Large 14 Random 17 Small 18 Large 20 Random 18 Small 18 Large 15 Random 16	Large 19 Random 17 26.2 (0.6) Small 19 Large 17 Random 16 24.1 (1.5) Small 20 Large 14 Random 17 27.8 (3.1) Small 18 Large 20 Random 18 24.9 (1.5) Small 18 Large 15 Random 16 25.7 (0.7)	Large 19 9.3 (1.2) Random 17 26.2 (0.6) 6.9 (1.2) Small 19 4.6 (1.1) Large 17 7.7 (1.5) Random 16 24.1 (1.5) 5.8 (1.4) Small 20 4.5 (0.9) Large 14 7.7 (1.4) Random 17 27.8 (3.1) 6.1 (1.2) Small 18 4.9 (0.8) Large 20 5.5 (1.1) Random 18 24.9 (1.5) 5.4 (1.0) Small 18 4.2 (0.7) Large 15 8.5 (0.9) Random 16 25.7 (0.7) 6.6 (1.1)

^{*} Herds described in Table 1; piglet categories and selection process described in Table 2. All values are presented as mean (SD). Hb converted from mmol/L to the conventional unit, g/L.

Prevalence of Low, Medium, and High Hb status

Hemoglobin status was low in four Large (4.71%), three Random (3.57%), and three Small piglets (3.23%); moderate in 14 Large (16.47%), 13 Random (15.48%), and 13 Small piglets (13.98%); and high in 67 Large (78.82%), 68 Random (80.95%), and 77 Small piglets (82.80%). Hemoglobin status category did not differ among the three piglet size groups (Large, P = .92; Random, P = .88; and Small, P = .81).

Discussion

The present study showed that several hematological and hematochemical parameters differed significantly among piglets of different sizes (Small, Random, or Large) at weaning. The Large piglets were at higher risk of developing iron deficiency than Small piglets, as determined by MCHC, MCH, RDW, MCVr, CHCMr, CHr, RDWr, serum iron, TIBC, and TfS assays, which are reliable indicators of iron deficiency in either human or pig studies. 7.13,17-20

Neither Hb concentration nor the prevalence of Low (anemia), Medium, and High Hb concentration differed among the three piglet sizes, suggesting that assessment of iron status using Hb concentration alone may underestimate the iron requirement of piglets. It has been claimed that the sensitivity and specificity of Hb for diagnosis of iron deficiency anemia are low.⁶

Three stages of iron deficiency exist.²¹ In the first stage, total body iron is diminished but

 $^{^{}abc}$ Body weight means with different superscripts differ significantly (P < .001; ANOVA).

SD = standard deviation.

Among the 20 piglets selected in each category in each herd, four were removed from the study. Hemolyzed blood samples from 33 piglets were discarded, and one erroneous Hb value was removed.

SD = standard deviation; Hb = hemoglobin.

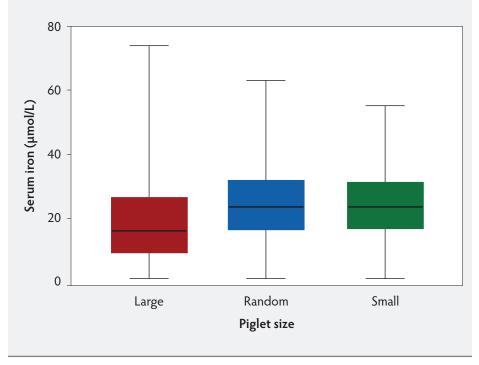
erythropoiesis and synthesis of Hb are not affected. In the second stage, the iron supply to the erythropoietic bone marrow is inadequate, but Hb synthesis is not affected. In the third stage, the iron supply is insufficient to maintain a normal Hb concentration. The initial stages of iron deficiency may be overlooked when Hb concentration alone is used as an indicator of iron status. Large piglets in the current study were probably in the second stage of iron deficiency, as the average Hb concentration did not differ in piglets of different sizes, but other indicators of current erythropoiesis did differ in the Large piglets compared to the other two size categories. The decline in Hb concentration may be noticed only in the third stage of iron deficiency, as iron is shunted from other iron pools to Hb.²² Inhibition or impairment of some metabolic processes may occur long before Hb formation becomes adversely affected.23

Measures of mature erythrocyte indices in the current study (eg, Hb, RBC, MCV, HCT) did not indicate a difference in iron status among the three piglet sizes. This is in agreement with other studies, ^{13,24} which suggests that these indices are not sensitive indicators of early iron-deficient erythropoiesis because erythrocytes have a slow turnover rate (85 days).

Reticulocyte red cell distribution width measures variation in RBC size ¹⁶ and is considered one of the reliable parameters indicating iron deficiency. The RDW increases during iron deficiency, ¹⁴ and it is therefore noteworthy that the Large piglets in our study had higher RDW than did the Small piglets. Although the average RDWs in the three sizes of piglets were within the reference range (16.4% to 32.3%), ²⁵ it should be noted that the reference ranges vary greatly by breed, age, sex, season, physiological status, and management factors. ¹⁶

Most of the reticulocyte indices (CHr, MCVr, CHCMr and RDWr) in the current study differed among the piglet sizes. Measures of reticulocyte indices, such as CHr, are sensitive indicators of early iron-deficient erythropoiesis, reflecting recent bone marrow activity, because of the very short life span of a reticulocyte (4 days). ^{12,13} A study has demonstrated that in piglets injected with 200 mg iron dextran at the age of 3 days, the MCV and MCH declined significantly at the age of 22 days; however, Hb, hematocrit, and RBC did

Figure 1: Box plot showing serum iron concentration in Large (n = 99), Random (n = 93), and Small piglets (n = 100) from five Danish farrow-finish sow herds. Assignment of piglets to groups is described in Table 2.



not fall, demonstrating the low sensitivity of these indices in detecting impaired erythropoiesis. The iron concentration in blood plasma declined even earlier, ie, at the age of 16 days. The same author found that the sensitivity of MCV and MCH to impaired erythropoiesis was comparable with reticulocyte indices; however, this was not confirmed in the present study.

Serum iron is the amount of circulating iron that is bound to transferrin, and TIBC is the capacity of plasma proteins to bind iron. 19 Transferrin saturation reflects the percentage of transferrin iron-binding sites that are occupied. 16 During iron deficiency, serum iron declines with a rise in TIBC, resulting in low TfS values.²⁰ The Large piglets in the current study had lower serum iron, higher TIBC, and lower TfS than did Small piglets, which probably reflects higher risk of iron deficiency in large piglets. Nevertheless, the average serum iron concentration and TIBC in the three piglet sizes was within the normal range.²⁶ However, reference values for TfS were not found for piglets and nurseryage pigs.

The current study found higher numbers and percentages of neutrophils and basophils in larger piglets than in smaller ones. Iron has important effects on both granulocyte functions and counts; however,

the exact role is still obscure. Increased neutrophil count during iron deficiency is believed to be associated with changes in apoptotic response,²⁷ lower oxidative burst, and oxidant product synthesis,²⁸ resulting in increased neutrophil lifespan.

A previous study⁵ has also demonstrated that iron injections of 200 mg at birth depleted at 17 days of age in heavier and fast-growing piglets. However, the optimum dosage and timing of injectable iron to ensure adequacy is a matter of discussion. Iron status may be improved by additional dosing during the suckling period. In the present study, only one injection at day 3 to 4 was administered.

Iron is involved in the transport of oxygen, in electron transfer, in synthesis of DNA, in oxidation reactions, and in many other processes maintaining normal structure and function of cells.²⁴ Hence, possible clinical and subclinical effects caused by iron deficiency or other hematological abnormalities in piglets in the modern swine industry need to be addressed.

Table 4: Hematological and hematochemical parameters in Large, Random, and Small piglets*

		Large piglet			Random piglet		Small piglet	
Parameters	Unit	n	Mean (SD)	n	Mean (SD)	n	Mean (SD)	Р
RBC	10 ¹² /L	85	6.52 (0.57)	85	6.34 (0.69)	93	6.38 (0.61)	.14†
Hct	L/L	85	0.38 (0.04)	85	0.38 (0.04)	93	0.38 (0.03)	.73‡
Hb	g/L	85	119.64 (15.50)	84	121.50 (15.00)	93	121.50 (13.20)	.75‡
MCV	fL	85	58.71 (6.60)	85	60.47 (5.54)	93	59.9 (5.44)	.28‡
MCHC	g/L	85	19.21 (2.06) ^a	85	19.67 (0.95) ^b	93	19.76 (0.75) ^b	< .001‡
MCH	pg/cell	85	1.13 (0.18) ^a	85	1.19 (0.11) ^b	93	1.18 (0.11) ^b	.03‡
Platelets	10 ⁹ /L	85	221.09 (115.18)	85	215.41 (159.37)	93	208.41 (109.75)	.73†
MPV	fL	85	11.76 (2.51)	85	12.23 (2.40)	93	11.56 (2.48)	.18‡
WBC	10 ⁹ /L	85	14.98 (5.15)	85	14.05 (4.43)	93	14.64 (4.57)	.44†
RDW	%	85	19.56 (3.42) ^a	85	18.23 (2.97) ^b	93	17.56 (2.37) ^b	< .001‡
HDW	mmol/L	85	1.37 (0.13)	85	1.34 (0.11)	93	1.35 (0.11)	.40‡
M	10 ⁹ /L	85	0.55 (0.28)	85	0.53 (0.25)	93	0.48 (0.25)	.13†
Monocytes	%	85	4.16 (1.26) ^a	85	4.36 (1.41) ^a	93	3.79 (1.46) ^b	.01‡
1	10 ⁹ /L	85	7.09 (3.31) ^a	85	6.30 (2.68) ^{ab}	93	6.0 (2.63) ^b	.04†
Lymphocytes	%	85	53.04 (10.91) ^a	85	51.03 (10.59) ^a	93	46.89 (11.06) ^b	< .001†
N1. 11.1.	10 ⁹ /L	85	4.76 (2.32) ^a	85	4.68 (2.06) ^a	93	5.52 (2.46) ^b	.01†
Neutrophils	%	85	36.68 (10.66) ^a	85	38.61 (10.15) ^a	93	43 (10.80) ^b	< .001†
Fig. 1. 1. 1. 1. 1.	10 ⁹ /L	85	0.52 (0.24)	85	0.51 (0.28)	93	0.57 (0.36)	.41†
Eosinophils	%	85	4.23 (2.11)	85	4.24 (2.09)	93	4.7 (3.12)	.65‡
Danasahila	10 ⁹ /L	85	0.14 (0.15) ^a	85	0.10 (0.09) ^{ab}	93	0.09 (0.10) ^b	.02‡
Basophils	%	85	0.96 (0.69) ^a	85	0.80 (0.49) ^a	93	0.69 (0.49) ^b	< .01‡
D-4:l	10 ⁹ /L	85	311.62 (109.28)	85	309.04 (135.65)	93	287.06 (128.08)	.23†
Reticulocytes	%	85	4.81 (1.73)	85	4.91 (2.35)	93	4.54 (2.12)	.47‡
MCVr	fL	85	65.28 (6.70) ^a	85	68.14 (5.41) ^b	93	68.14 (5.47) ^b	< .01‡
CHCMr	mmol/L	85	15.69 (0.50) ^a	85	15.89 (0.56) ^b	93	16.03 (0.48) ^c	< .001‡
CHr	fmol	85	1.01 (0.10) ^a	85	1.07 (0.08) ^b	93	1.08 (0.08) ^b	< .001‡
RDWr	%	85	15.93 (2.60) ^a	85	15.43 (2.67) ^b	93	15.03 (1.84) ^b	< .01‡
HDWr	mmol/L	85	1.94 (0.29)	85	1.88 (0.26)	93	1.86 (0.26)	.14‡
Serum iron	μmol/L	99	19.89 (13.5) ^a	93	25.11 (12.38) ^b	100	24.56 (11.58) ^b	< .01‡
TIBC	μmol/L	99	88.63 (17.32) ^a	93	82.02 (18.35) ^b	100	72.49 (19.42) ^c	<.001‡
TfS	%	99	24.32 (17.22) ^a	93	33.14 (18.20) ^b	100	37.01 (19.94) ^c	< .01‡

^{*} Study design described in Tables 1 and 2.

[†] ANOVA.

[‡] Kruskal-Wallis test.

 $^{^{}abc}$ Within a row, means with different superscripts differ significantly (P < .05).

SD = standard deviation; RBC = red blood cell count; Hct = hematocrit; Hb = hemoglobin; MCV = mean corpuscular volume; MCHC = mean cell hemoglobin concentration; MCH = mean corpuscular hemoglobin; MPV = mean platelet volume; WBC = white blood cells; RDW = red blood cell distribution width; HDW = hemoglobin distribution width; MCVr = reticulocyte cellular volume; CHCMr = mean reticulocyte corpuscular hemoglobin concentration; CHr = reticulocyte hemoglobin content; RDWr = reticulocyte red cell distribution width; HDWr = reticulocyte hemoglobin distribution width; TIBC = total iron-binding capacity; TfS = transferrin saturation.

Table 5: Normal hematological values reported for piglets*

Parameters	Unit	Minimum	Maximum	Mean	References
RBC	10 ¹² /L	4.4	5.3	4.9	16
Hct*	L/L	0.35	0.40	0.37	16
Hb*	g/L	90	112	102	16
MCV	fL	70	82	76	16
MCHC*	g/L	16.97	20.14	18.78	25
MCH*	pg/cell	0.68	1.13	0.91	25
Platelets*	10 ⁹ /L	138	909	540.7	25
WBC*	10 ⁹ /L	6.2	10.5	7.7	16
RDW	%	16.1	33.3	24.4	25
HDW*	g/L	NA	NA	1.55	26
Monocytes*	10 ⁹ /L	0.23	1.46	0.69	25
	%	2	7	4.3	16
Lymphocytes*	10 ⁹ /L	4.04	15.74	8.67	25
	%	55	82	66.8	16
Neutrophils*	10 ⁹ /L	1.19	15.74	5.66	25
	%	13.5	39.5	25.7	16
Eosinophils*	10 ⁹ /L	0.058	0.574	0.219	25
	%	0	2	0.8	16
Basophils*	10 ⁹ /L	0.011	0.151	0.053	25
	%	0	0.5	0.05	16
Reticulocytes	%	9	13	10.6	16
Serum iron	μmol/L	5	83	33	26
TIBC	μmol/L	59	141	94	26

Hb converted to the conventional unit, g/L. For ease of comparison, units of all other parameters were converted to match the study data in Table 4.

RBC = red blood cell count; Hct = hematocrit; Hb = hemoglobin; MCV = mean corpuscular volume; MCHC = mean cell hemoglobin concentration; MCH = mean corpuscular hemoglobin; WBC = white blood cell count; RDW = red blood cell distribution width; HDW = hemoglobin distribution width; TIBC = total iron-binding capacity; NA = not available.

Implications

- Large piglets in a litter at weaning are at higher risk of developing iron deficiency anemia than are smaller piglets.
- Alternative hematological indices might serve as better early indicators of iron deficiency than traditionally used Hb concentration.

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Conflict of interest

None reported.

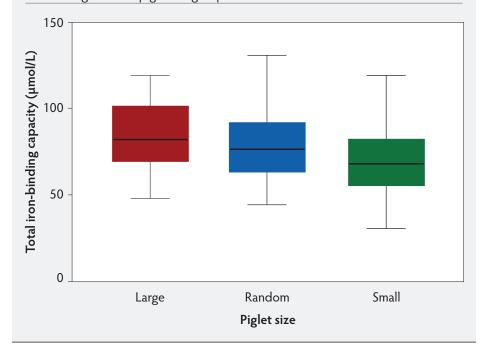
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References

- 1. Douglas TA, Renton JP, Watts C, Ducker HA. Placental transfer of iron in the sow (*Sus domesticus*). *Comp Biochem Physiol A Physiol*. 1972;43:665–671.
- 2. Benkhedda K, L'abbé MR, Cockell KA. Effect of calcium on iron absorption in women with marginal iron status. *Br J Nutr.* 2010;103:742–748.
- 3. Quiniou N, Dagorn J, Gaudre D. Variation of piglets' birth weight and consequences on subsequent performance. *Livest Prod Sci.* 2002;78:63–70.
- 4. Wolf J, Žáková E, Groeneveld E. Within-litter variation of birth weight in hyperprolific Czech Large White sows and its relation to litter size traits, stillborn piglets and losses until weaning. *Livest Sci.* 2008;115:195–205.
- 5. 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;89:4068–4080.
- 6. Cook JD. Diagnosis and management of irondeficiency anaemia. *Best Pract Res Clin Haematol*. 2005;18:319–332.

Figure 2: Box plot showing total iron-binding capacity (μ mol/L) in Large (n = 99), Random (n = 93), and Small piglets (n = 100) from five Danish farrow-finish sow herds. Assignment of piglets to groups is described in Table 2.



- 7. Svoboda M, Ficek R, Drabek J. Reticulocyte indices in the diagnosis of iron deficiency in suckling piglets. *Bull Vet Inst Pulawy*. 2008;52:125–130.
- 8. Andrews GA, Chavey PS, Smith JE. Enzymelinked immunosorbent assay to measure serum ferritin and the relationship between serum ferritin and nonheme iron stores in cats. *Vet Pathol.* 1994;31:674–678.
- 9. Furugouri K, Miyata Y, Shijimaya K. Ferritin in blood serum of dairy cows. *J Dairy Sci.* 1982;65:1529–1534.
- 10. Ilić V, Petakov M, Stojanović N, Jovčić G, Bugarski D, Grbović T, Božić T, Kovačević-Filipović M. Relationship between total iron binding capacity and transferrin concentration in neonatal piglets treated with iron-dextran. *Acta Vet Brno.* 2006;56:235–242.
- 11. Lipschitz DA, Cook JD, Finch CA. A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med.* 1974;290:1213–1216.

- 12. Macdougall IC, Cavill I, Hulme B, Bain B, McGregor E, McKay P, Sanders E, Coles GA, Williams JD. Detection of functional iron deficiency during erythropoietin treatment: a new approach. *Brit Med J* . 1992;304:225–226.
- 13. Mast AE, Blinder MA, Dietzen DJ. Reticulocyte hemoglobin content. *Am J Hematol.* 2008;83:307–310.
- 14. McClure S, Custer E, Bessman JD. Improved detection of early iron deficiency in nonanemic subjects. *JAMA*. 1985;253:1021–1023.
- 15. Smith J, Moore K, Boyington D, Pollmann DS, Schoneweis D. Serum ferritin and total ironbinding capacity to estimate iron storage in pigs. *Vet Pathol.* 1984;21:597–600.
- 16. Thorn CE. Hematology of the pig. In: Weiss DJ, Wardrop JK, eds. *Schalm's Veterinary Hematology*. 6th ed. Ames, Iowa: Wiley-Blackwell; 2010:843.

- 17. Qurtom HA, Al-Saleh QA, Lubani MM, Hassanein A, Kaddoorah N, Qurtom MA, Al-Sheikh T. The value of red cell distribution width in the diagnosis of anaemia in children. *Eur J Pediatr*. 1989;148:745–748.
- 18. Aulakh R, Sohi I, Singh T, Kakkar N. Red cell distribution width (RDW) in the diagnosis of iron deficiency with microcytic hypochromic anemia. *Indian J Pediatr*. 2009;76:265–268.
- 19. Szőke D, Panteghini M. Diagnostic value of transferrin. *Clin Chim Acta*. 2012;413:1184–1189.
- 20. Hawkins RC. Total iron binding capacity or transferrin concentration alone outperforms iron and saturation indices in predicting iron deficiency. *Clin Chim Acta*. 2007;380:203–207.
- 21. Hastka J, Lasserre JJ, Schwarzbeck A, Hehlmann R. Central role of zinc protoporphyrin in staging iron deficiency. *Clin Chem.* 1994;40:768–773.
- 22. Smith J. Iron metabolism and its disorders. In: Kaneko JJ, Harvey J, Bruss M, eds. *Clinical Biochemistry of Domestic Animals*. 5th ed. Burlington, Massachusetts: Academic Press; 1997:223–239.
- 23. Svoboda M, Drabek J. Iron deficiency in suckling piglets: etiology, clinical aspects and diagnosis. *Folia Veterinaria*. 2005;49:104–111.
- 24. Morris CJ, Earl JR, Trenam CW, Blake DR. Reactive oxygen species and iron a dangerous partnership in inflammation. *Int J Biochem Cell Biol.* 1995;27:109–122.
- 25. Cooper CA, Moraes LE, Murray JD, Owens SD. Hematologic and biochemical reference intervals for specific pathogen free 6-week-old Hampshire-Yorkshire crossbred pigs. *J Anim Sci Biotechnol*. 2014;5:1–6.
- 26. Egeli A, Framstad T, Morberg H. Clinical biochemistry, hematology and body weight in piglets. *Acta Vet Scand.* 1998;39:381–393.
- 27. Paino IM, Miranda JC, Marzocchi-Machado CM, Cesarino, EJ, de Castro FA, de Souza AM. Phagocytosis, oxidative burst, and produced reactive species are affected by iron deficiency anemia and anemia of chronic diseases in elderly. *Biol Trace Elem Res.* 2009;129:116–125.
- 28. Berrak SG, Angaji M, Turkkan E, Canpolat C, Timur C, Eksioglu-Demiralp E. The effects of iron deficiency on neutrophil/monocyte apoptosis in children. *Cell Prolif.* 2007;40:741–754.

