ORIGINAL RESEARCH **PEER REVIEWED**

Effects of different parenteral iron treatment regimens on hematology characteristics, serum concentrations of hepcidin, and growth performance in pigs fed nursery diets supplemented with copper

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Summary

Objective: To determine the effects of iron treatments on hematology, hepcidin, and growth in weaned pigs fed copper-supplemented diets.

Materials and methods: Pigs were allocated to a 3 × 2 factorial arrangement of treatments (4 pens/treatment combination, 3 pigs/pen) with factors being intramuscular iron (200 mg at birth; 100 mg at birth and weaning [22.4 days of age]; or 100 mg at birth and 14 days of age) and dietary copper (14 [control] or 250 ppm [supplemented]). Blood was sampled at days 0, 7, and 49 post weaning.

Results: Pigs receiving 100 mg iron at birth and weaning, but not pigs in the other groups, had hemoglobin concentrations consistent with iron deficiency at day 0 (iron treatment × day, $P < .001$). For pigs receiving 100 mg iron at birth and 14 days of age, hepcidin concentrations were greater in control pigs than copper-supplemented pigs (iron treatment × diet, $P = .06$). A diet × day interaction ($P = .07$) existed for hepcidin, with concentrations greater in control vs copper-supplemented pigs on day 49. Pigs receiving iron at day 14 of age had the greatest ($P = .01$) weaning weights. Gain from day 0 to 7 was enhanced ($P = .03$) by 250 ppm copper but nursery performance (day 0-49) was unaffected by iron treatment.

Implications: Pigs receiving 100 mg iron at birth were iron deficient at weaning. Treatment with iron at 14 days of age could improve weaning weights and prevent iron deficiency at weaning. Age-related increases in hepcidin were decreased by additional copper supplementation.

Keywords: swine, performance, iron, copper, hepcidin

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Resumen - Efectos de diferentes regímenes de tratamiento con hierro parenteral sobre las características hematológicas, las concentraciones séricas de hepcidina, y el rendimiento del crecimiento en cerdos alimentados con dietas de destete suplementadas con cobre

Objetivo: Determinar los efectos de los tratamientos con hierro sobre la hematología, la hepcidina, y el crecimiento en cerdos destetados alimentados con dietas suplementadas con cobre.

Materiales y métodos: Los cerdos fueron asignados en un acomodo factorial de tratamientos 3 × 2 (4 corrales/combinación de tratamiento, 3 lechones/corral) con factores que fueron hierro intramuscular (200 mg al nacimiento; 100 mg al nacimiento y al destete [22.4 días de edad]; o 100 mg al nacimiento y 14 días de edad) y cobre dietético (14 [control] o 250 ppm [suplementado]). Se tomaron muestras de sangre los días 0, 7, y 49 después del destete.

Resultados: Los cerdos que recibieron 100 mg de hierro al nacimiento y al destete, pero no los cerdos de los otros grupos tenían concentraciones de hemoglobina compatibles con deficiencia de hierro el día 0 (tratamiento con hierro por día, $P < .001$). En los cerdos que recibieron 100 mg de hierro al nacimiento y a los 14 días de edad, las concentraciones de hepcidina fueron mayores en los cerdos control que en los cerdos suplementados con cobre (tratamiento con hierro × dieta, $P = .06$). Existió una interacción de dieta por día ($P = .07$) para la hepcidina, con concentraciones mayores en el control que en los cerdos suplementados con cobre en el día 49. Los cerdos que recibieron hierro en el día 14 de edad tuvieron los mayores ($P = .01$) pesos al destete. La ganancia del día 0 al 7 mejoró ($P = .03$) con 250 ppm de cobre, pero el rendimiento del destete (día 0-49) no se vio afectado por el tratamiento con hierro.

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Implicaciones: Los cerdos que recibieron 100 mg de hierro al nacimiento tenían deficiencia de hierro al destete. El tratamiento con hierro a los 14 días de edad podría mejorar los pesos al destete y prevenir la deficiencia de hierro al destete. Los aumentos de hepcidina relacionados con la edad se redujeron con suplementos adicionales de cobre.

Résumé - Effets de différents régimes de traitement parentéral au fer sur les caractéristiques hématologiques, les concentrations sériques d'hepcidine, et les performances de croissance chez les porcs nourris avec des régimes de pouponnière enrichis en cuivre

Objectif: Déterminer les effets des traitements au fer sur l'hématologie, l'hepcidine, et la croissance chez des porcs sevrés nourris avec des régimes enrichis en cuivre.

Matériels et méthodes: Les porcs ont été répartis selon un arrangement factoriel 3 × 2 de traitements (4 enclos/combinaison de traitement, 3 porcs/enclos) les facteurs étant le fer intramusculaire (200 mg à la naissance; 100 mg à la naissance et au sevrage [22.4 jours d'âge]; ou 100 mg à la naissance et à 14 jours d'âge) et du cuivre alimentaire (14 [témoin] ou 250 ppm [supplémenté]). Du sang a été prélevé aux jours 0, 7, et 49 après le sevrage.

Résultats: Les porcs recevant 100 mg de fer à la naissance et au sevrage, mais pas les porcs des autres groupes, présentaient des concentrations d'hémoglobine compatibles avec une carence en fer au jour 0 (traitement au fer par jour, $P < .001$). Pour les porcs recevant 100 mg de fer à la naissance et à l'âge de 14 jours, les concentrations d'hepcidine étaient plus élevées chez les porcs témoins que chez les porcs supplémentés en cuivre (traitement au fer × alimentation, $P = .06$). Une interaction régime/jour ($P = .07$) existait pour l'hepcidine, avec des concentrations plus élevées chez les témoins que chez les porcs supplémentés en cuivre au jour 49. Les porcs recevant du fer au jour 14 avaient les poids au sevrage les plus élevés ($P = .01$). Le gain du jour 0 au jour 7 a été amélioré ($P = .03$) par 250 ppm de cuivre mais la performance en pouponnière (jour 0-49) n'a pas été affectée par le traitement au fer.

Implications: Les porcs recevant 100 mg de fer à la naissance présentaient une carence en fer au sevrage. Un traitement au fer à l'âge de 14 jours pourrait améliorer le poids au sevrage et prévenir la carence en fer au sevrage. Les augmentations d'hepcidine liées à l'âge ont été réduites par une supplémentation additionnelle en cuivre.

Iron deficiency anemia develops in suckling pigs unless exogenous iron is supplied early in life. On commercial sow farms, neonatal pigs are treated intramuscularly (IM) with iron dextran or gleptoferron, and doses of 100 to 200 mg have been used to prevent iron deficiency anemia.[1-3] Modern sows, however, produce large litters of pigs with capacity for rapid preweaning growth. Recent reports have indicated that despite iron supplementation given during the first week of life, many pigs, particularly the largest, fastest-growing animals in a litter, are anemic or iron deficient at weaning.[4-7] Pigs that are anemic at weaning are more susceptible to disease[8] and exhibit slower nursery growth rates.[5,9] The economic impact of iron deficiency on US pork production is estimated to be \$46 to \$335 million annually.[10]

Thus, there is renewed interest in the iron status of weaned pigs. This could be particularly important if growth-promoting levels of copper (200 to 250 ppm)[11-13] are used in nursery diets as pharmacological levels of copper may decrease iron absorption,[14,15] and perhaps exacerbate an iron deficient condition. Treatment with IM iron doses in excess of 200 mg could be toxic to some pigs,[16] encourage bacterial growth and susceptibility to infection,[17] or cause increased release of hepcidin, a hormone secreted by the liver that inhibits iron absorption.[18,19] Another strategy for increasing blood iron concentrations in nursery pigs is to alter the number and timing of injections of iron.[20] Thus, the objective of this study was to determine the effects of various iron treatment regimens on hematology, circulating hepcidin concentrations, and growth performance in nursery pigs fed copper-supplemented diets.

Animal care and use

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Virginia Tech (Blacksburg, VA).

Materials and methods

Study animals and housing

Eight Yorkshire × Landrace sows farrowed 75 Duroc-sired pigs, of which 72 pigs (38 males and 34 females) were used in this experiment. Three pigs were laid on by the sow prior to processing. Within 24 hours after birth, pigs were ear notched for identification, weighed, needle teeth were resected, and tails docked. No antibiotics were administered at processing or during the lactation and nursery periods. Pigs were transferred (n = 5) among litters so that sows were nursing an approximately equal number of pigs (9.0 ± 0.6 piglets). Boars were castrated at 7 days of age using a sterile scalpel. Pigs were not given creep feed during the suckling period.

The mean (SE) weaning age was 22.4 (0.2) days when pigs were moved to an environmentally controlled nursery facility. Each nursery pen measured 0.91 × 1.22 m over galvanized steel bar slats and contained a nipple drinker and a stainless-steel feeder with four feeding spaces.

Study design

Iron hydrogenated dextran (Iron-100; Durvet, Inc) was administered to pigs as an IM injection in the neck muscle behind the ear using a 20-gauge, 1.27-cm long needle. The following three iron treatment regimens were employed: 1) 200 mg iron at initial processing (birth); 2) 100 mg iron at birth and at weaning; and 3) 100 mg iron at birth and at 14 days of age.

Four blocks were created by placing 18 pigs in 6 pens (3 pigs/pen) in each block. Pens were balanced for body weight (BW), sex, and litter of origin. Pens within blocks were randomly allocated to a 3 × 2 factorial arrangement of treatments. The factors were 1) iron treatment (one of three treatments as previously described) and 2) level of dietary copper (14 [control] or 250 ppm [copper-supplemented]). There were four replicate pens per treatment combination (total of 24 pens). The sample size selected was needed to detect a 12.5% difference in performance with a coefficient of variation of 5%, assuming 80% power and a 5% significance level.

Experimental diets

Pigs were allowed *ad libitum* access to a phase feeding regimen with all diets meeting the requirements for the

Table 1: Composition of copper-supplemented and control diets fed to nursery pigs for 49 days*

	Dietary phase:	I	II	III
Ingredient, %	**Days fed relative to weaning:**	**0 - 7**	**8 - 21**	**22 - 49**
Ground corn		42.13	54.94	64.94
Soybean oil		3.00	3.00	3.00
Dried whey		25.00	10.00	0.00
Menhaden fish meal		4.00	2.00	0.00
Soycomil[†]		3.00	2.00	2.00
Soybean meal		19.85	24.90	26.65
Dicalcium phosphate		1.00	1.00	1.25
Calcium carbonate		0.70	1.00	1.00
Salt		0.20	0.20	0.20
Lysine-HCL		0.40	0.30	0.30
DL-methionine[‡]		0.12	0.06	0.06
Vitamin-trace mineral[§]		0.50	0.50	0.50
Copper sulfate or ground corn		0.10	0.10	0.10
Totals		**100.00**	**100.00**	**100.00**
Calculated analysis, %				
Crude protein		20.57	20.33	19.57
Lysine		1.53	1.37	1.27
Methionine		0.46	0.39	0.37
Calcium		0.88	0.83	0.74
Phosphorous		0.75	0.65	0.61

* Copper sulfate or control diets were prepared by mixing copper sulfate (Pestell Minerals and Ingredients) or ground corn, respectively, with basal diet consisting of the major portion of the ground corn and all other common ingredients. Control diets contained approximately 14.2 ppm copper, 113 ppm iron, and 113 ppm zinc.

[†] Archer Daniels Midland Co.

[‡] Rhodimet NP 99.

[§] ANS Swine Breeder Premix manufactured for Agri-Nutrition Services, Inc. Trace minerals in sulfate forms were in a polysaccharide complex.

various nutrients[21] and copper adjusted as previously indicated. For each of the three phases, a basal diet was first prepared containing most of the corn and all the common ingredients for each of the two experimental diets. Copper sulfate (Pestell Minerals and Ingredients) or an equal amount of ground corn was added to the basal diet to create the copper-supplemented or control diets, respectively (Table 1).

Data and sample collection and blood analyses

Pigs were weighed at weaning (day 0) and on days 7, 21, and 49 post weaning. Average daily gain (ADG) was determined for periods from day 0 to 7, day 8 to 21, day 22 to 49, and day 0 to 49. Feed additions were recorded so that for each period and the entire trial, average daily feed intake (ADFI) and the gain to feed ratio (G:F) could be determined.

A blood sample was collected from the barrow weighing closest to the mean pig weight in each pen at weaning (before receiving the weaning iron treatment), and at days 7 and 49 post weaning. The same pig was used on each collection day. Barrows were placed supine on a v-board and approximately 7 mL of blood was collected via jugular venipuncture (20-gauge, 2.54-cm long needle) into a vacutainer tube (Becton, Dickinson and Company) containing EDTA and a similar sized tube containing no anticoagulant.

Blood collected into tubes containing EDTA was used for hematology analyses using a Coulter Multisizer 3 cell counter (Beckman Coulter, Inc) by Animal Laboratory Services of the Virginia-Maryland College of Veterinary Medicine (Blacksburg, VA). The following hematological parameters were measured: number of red blood cells, reticulocytes, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets; hemoglobin concentration; hematocrit; mean corpuscular volume; mean corpuscular hemoglobin concentration; red blood cell distribution width; and mean platelet volume. Blood sample tubes containing no additive were allowed to clot for 24 hours at 4°C and serum was harvested following

30 minutes of centrifugation at 1820*g*. Serum concentrations of hepcidin were determined using a sandwich enzyme-linked immunoabsorbent assay kit (LS-F11619; LifeSpan BioSciences, Inc). Intra-assay coefficient of variation was 10% and assay sensitivity was 0.78 ng/mL.

Statistical analyses

Data were subjected to analysis of variance using the mixed-models procedure of SAS (SAS Institute Inc). Body weights, ADG, ADFI, and G:F were analyzed using a model that included iron treatment, diet, and iron treatment by diet interaction as possible sources of variation. Block was included as a random variable. Birth weight served as a covariate for BW at weaning (day 0) and BW at day 0 served as a covariate for BW at days 7, 21, and 49 post weaning. Pen was the experimental unit.

A repeated measures model was used for analyzing hematological characteristics and hepcidin. The model included iron treatment, diet, day, and all possible two- and three-way interactions as possible sources of variation. Block was included as a random variable and the individual pig was the experimental unit. Individual means were compared using the LSMEANS option of PROC MIXED and were adjusted using the Tukey-Kramer procedure. Effects were considered statistically significant at $P < .05$ with trends for significance at $P \le .10$.

Results

Hematology characteristics

Table 2 reports hematology characteristics in nursery pigs as affected by the main effects of iron treatment, diet, and day post weaning. There were no effects of iron treatment by diet by day post weaning or iron treatment by diet for any hematology measure. The concentration of red blood cells ($P = .06$), hemoglobin concentrations ($P < .001$), hematocrit ($P < .001$), mean corpuscular volume ($P < .001$), mean corpuscular hemoglobin concentration ($P < .001$), red blood cell distribution width ($P < .001$), and reticulocyte percentage ($P = .01$) and number ($P = .03$) were affected by iron treatment by day post weaning (Figures 1 and 2A). Red blood cell concentration tended to increase ($P = .06$) from weaning to day 7 post weaning and then remained similar to day 49 in pigs receiving 100 mg iron at birth and weaning. In the other two iron groups, red blood cell concentrations were similar across days. On day 0, hemoglobin ($P < .001$), hematocrit ($P < .001$), mean corpuscular volume ($P < .001$), and mean corpuscular hemoglobin ($P = .02$) in pigs receiving 100 mg iron at birth and weaning were less compared with pigs from the other two iron groups. In contrast, red blood cell distribution width and the number and percentage of reticulocytes, were greater in pigs receiving 100 mg iron at birth and weaning compared to the other two iron groups on both day 0 ($P = .002$, $P = .03$, and $P = .07$, respectively) and day 7 ($P = .02$, $P = .03$, and $P = .03$, respectively). The injection of 100 mg iron in the iron-deficient pigs at weaning caused hemoglobin concentrations, hematocrit, and mean corpuscular volume to increase to normal levels by day 7 post weaning. However, these pigs had lower mean corpuscular hemoglobin and greater red blood cell distribution width and number and percentage of reticulocytes at 7 days post weaning than pigs in the other two iron groups.

There were tendencies for effects of diet by day post weaning for mean corpuscular volume ($P = .06$) and mean corpuscular hemoglobin concentrations ($P = .08$; Figures 3A and B). Mean corpuscular volume in pigs fed the control diet was similar on day 0 and day 7 and tended ($P = .06$) to increase from day 7 to day 49 post weaning. In contrast, mean corpuscular volume was similar among days in 250 ppm copper-fed pigs. Mean corpuscular hemoglobin concentrations in 250 ppm copper-fed pigs tended to be greater ($P = .08$) on day 49 versus day 0, with day 7 having an intermediate value not different from the other two days. In contrast, mean corpuscular hemoglobin concentrations in control pigs were similar across days. Finally, reticulocyte concentration was greater in pigs fed the copper-supplemented diet compared to controls (diet, $P = .03$; Table 2).

Eosinophil concentration was affected ($P = .05$) by an interaction of iron treatment and day (Figure 2B). For pigs receiving 100 mg iron at birth and 100 mg at either day 14 of age or weaning, eosinophil concentrations were greater ($P = .05$) on day 49 than on days 0 and 7; however, they were similar across days in pigs receiving 200 mg iron at birth. There was also a tendency for an effect of diet by day for eosinophil concentrations ($P = .06$; Figure 3C). Eosinophil concentrations in pigs from both dietary treatment groups were similar on days 0 and 7, and then increased to day 49 post weaning; however, concentrations on day 49 tended to be greater ($P = .06$) in copper-supplemented pigs versus controls.

Overall, white blood cell concentrations, as well as the various populations of white blood cells, were affected by day post weaning. White blood cell concentrations were greater ($P < .001$) on day 49 than on day 0 or day 7, which did not differ. Concentrations of lymphocytes increased ($P < .001$) from day 0 to day 7, and then remained similar to day 49 post weaning. Monocyte concentrations ($P < .001$) increased from day 0 to day 7 and further increased to day 49. The concentration of basophils ($P = .04$) was greater on day 49 compared to day 0 post weaning, with values on day 7 being intermediate and not different from the other two days.

The concentrations of platelets ($P = .09$) and mean platelet volume ($P = .09$) tended to be affected by iron treatment by day (Figures 2C and D). Platelet concentration tended ($P = .09$) to be greater on day 0 versus day 7 or day 49 post weaning in pigs receiving 200 mg iron at birth, decreased from day 0 to day 7, and further decreased to day 49 in pigs receiving 100 mg iron at birth and weaning. Platelet concentration in pigs receiving 100 mg iron at birth and day 14 of age tended to be less on day 49 than either day 0 or day 7. Mean platelet volume tended to increase ($P = .09$) from day 0 to 7 and further increased to day 49 in pigs receiving 100 mg iron at birth and 100 mg at either day 14 of age or weaning. In contrast, mean platelet volume increased from day 0 to day 7 and then remained similar to day 49 post weaning in pigs receiving 200 mg iron at birth. There was also an effect of diet by day post weaning for platelet concentrations ($P = .03$; Figure 3D). For pigs fed the control diets, platelet concentration decreased ($P = .03$) from day 0 to day 7 and further decreased ($P = .03$) to day 49 post weaning. In copper-supplemented pigs, however, platelet concentrations decreased ($P = .03$) from day 0 to 7 and remained similar ($P = .21$) to day 49.

Serum hepcidin concentrations

There was an effect of day post weaning ($P < .001$) on hepcidin concentrations on days 0, 7, and 49, which were 16.5, 44.0, and 177.0 ng/mL, respectively. Hepcidin concentrations tended to be affected by iron treatment ($P = .06$) with pigs receiving 100 mg at birth and day 14 having the greatest concentration (88.8 ng/mL) and

Table 2: Hematology characteristics in pigs receiving different iron treatment regimens and fed control or copper-supplemented (250 ppm) diets during the 49-day nursery phase of production

	Iron treatment[*,‡]					Diet				Day post-weaning				
Characteristics	1 (n = 8)	2 (n = 8)	3 (n = 8)	SE	*P*[†]	Control (n = 12)	Copper (n = 12)	SE	*P*[†]	0 (n = 24)	7 (n = 24)	49 (n = 24)	SE	*P*[†]
Red blood cells, × 10^{6}/μL[‡]	6.98	7.21	7.17	0.24	.60	7.11	7.13	0.20	.96	6.89^{a}	7.29^{b}	7.18^{a,b}	0.13	.002
Hemoglobin, g/dL[‡]	12.62^{a,b}	11.71^{a}	12.64^{a}	0.39	.03	12.28	12.37	0.31	.76	11.88^{a}	12.33^{a,b}	12.76^{b}	0.24	.005
Hematocrit, %[‡]	42.15	40.37	42.75	1.13	.10	41.68	41.83	0.91	.87	40.49^{a}	41.78^{a,b}	42.99^{b}	0.86	.02
Mean corpuscular volume, fL[‡,§]	60.48^{a}	56.2^{b}	59.80^{a}	1.15	.001	58.66	58.99	0.93	.72	58.79^{a}	57.43^{b}	60.25^{c}	0.60	< .001
Mean corpuscular hemoglobin, g/dL[‡,§]	29.93^{a}	28.94^{b}	29.58^{a,b}	0.30	.008	29.44	29.53	0.25	.73	29.24	29.53	29.68	0.21	.10
Red blood cell distribution width, %[‡]	16.77^{a}	21.06^{b}	17.82^{a}	1.12	.001	18.69	18.41	0.91	.76	20.12	19.48	16.05	0.41	< .001
Reticulocytes, %[‡]	3.33^{a}	4.87^{b}	3.37^{a}	0.36	< .001	3.63	4.08	0.25	.13	5.65	3.25	2.67	0.34	< .001
Reticulocytes, × 10^{3}/μL[‡]	229.68^{a}	342.56^{b}	237.57^{a}	20.00	< .001	251.54^{a}	288.34^{b}	16.65	.03	385.31^{a}	234.83^{a,b}	189.67^{b}	24.23	< .001
White blood cells, × 10^{3}/μL	17.64	16.18	15.74	1.30	.53	16.79	16.25	1.04	.71	14.01^{a}	15.74a	19.79^{b}	1.40	< .001
Neutrophils, × 10^{3}/μL	6.96	6.36	5.83	1.29	.68	6.54	6.23	0.75	.77	6.54	5.57	7.04	0.71	.10
Lymphocytes, × 10^{3}/μL	9.42	8.60	8.63	0.90	.58	9.04	8.74	0.73	.68	6.72^{a}	8.95^{b}	10.99^{b}	0.89	< .001
Monocytes, × 10^{3}/μL	0.84	0.74	0.71	0.11	.44	0.79	0.74	0.09	.55	0.40^{a}	0.75^{b}	1.13^{c}	0.12	< .001
Eosinophils, × 10^{3}/μL[‡,§]	0.35	0.36	0.40	0.07	.78	0.32	0.42	0.06	.06	0.22^{a}	0.25^{a}	0.65^{b}	0.05	< .001
Basophils, × 10^{3}/μL	0.11	0.10	0.08	0.02	.31	0.10	0.10	0.02	.94	0.07^{a}	0.10^{a,b}	0.12^{b}	0.02	.04
Platelets, × 10^{3}/μL[‡,§]	296.61	365.22	315.07	36.69	.16	328.22	323.05	29.68	.86	499.00^{a}	308.73^{b}	169.17^{c}	28.28	< .001
Mean platelet volume, fL[‡]	10.11	10.48	10.53	0.37	.46	10.49	10.25	0.30	.42	8.06^{a}	10.44^{b}	12.60^{c}	0.36	< .001

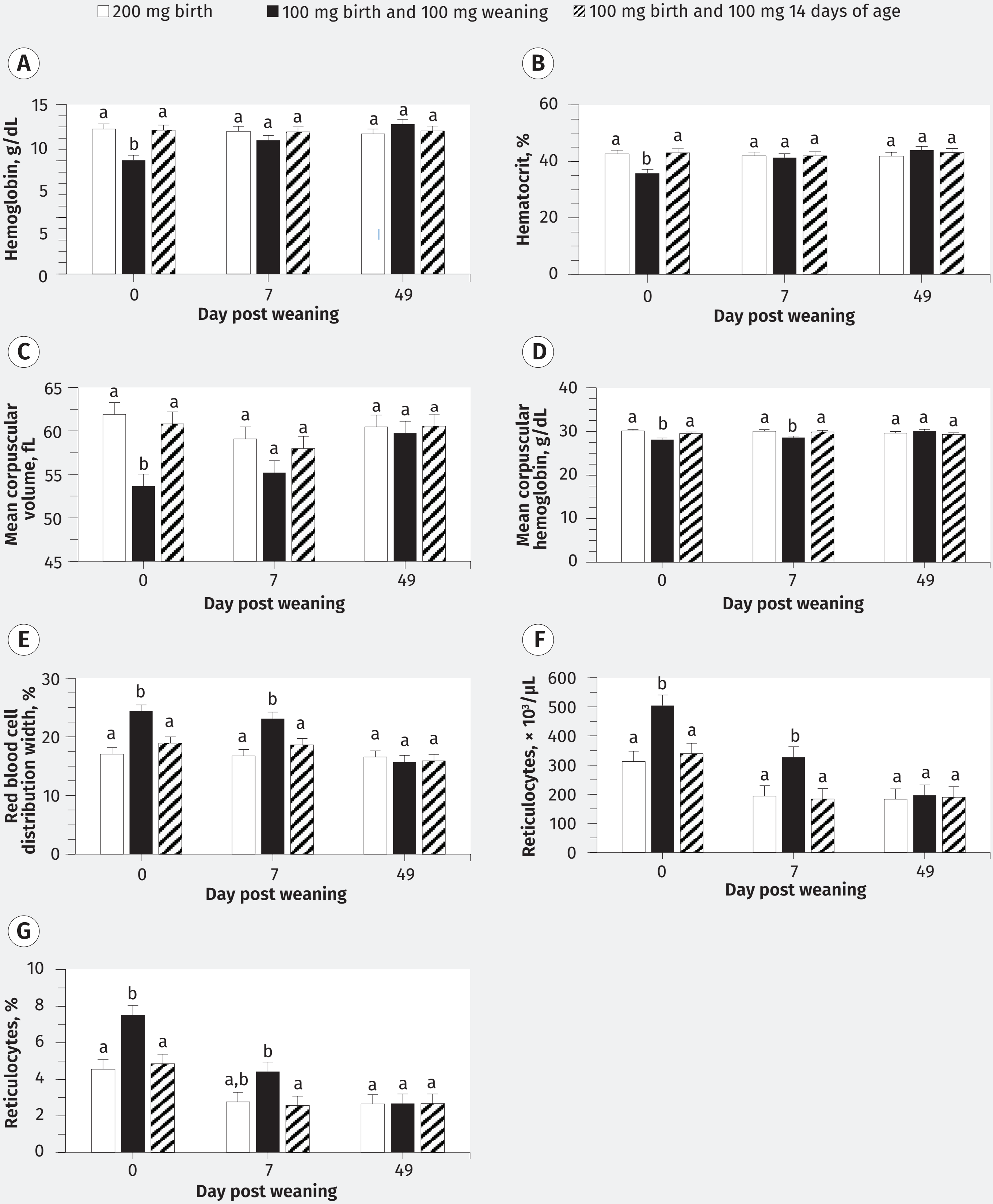
[*] Treatment 1 = 200 mg iron at birth; Treatment 2 = 100 mg iron at birth and 100 mg iron at weaning (22.4 days of age); and Treatment 3 = 100 mg iron at birth and 100 mg iron at day 14 of age.

[†] Data were subjected to ANOVA. The model included iron treatment, diet, and day and all two- and three-way interactions as possible sources of variation. For main effects of treatment, and day, values within a row with different superscripts (^{a,b,c}) differ ($P < .05$).

[‡] Affected by an interaction, or tendency for an interaction, between iron treatment and day (red blood cells, $P = .06$; hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and red blood cell distribution width, $P < .001$; reticulocytes, %, $P = .01$; reticulocyte number, $P = .03$; eosinophils, $P = .05$; platelets, $P = .09$; and mean platelet volume, $P = .09$).

[§] Affected by an interaction, or tendency for an interaction, between diet and day post weaning (mean corpuscular volume, $P = .06$ mean corpuscular hemoglobin $P = .08$; eosinophils, $P = .06$; and platelets, $P = .03$).

Figure 1: Hematology characteristics of pigs receiving 200 mg of iron at birth, or 100 mg iron at both birth and day 14 of age, or 100 mg iron at both birth and weaning (22.4 days of age) by intramuscular injection and a control or copper-supplemented diet (14 or 250 ppm copper, respectively). A) Hemoglobin, B) hematocrit, C) mean corpuscular volume, D) mean corpuscular hemoglobin, E) red blood cell distribution width (*P* < .001), F) reticulocytes number (*P* = 0.03), and G) reticulocyte percentage (*P* = 0.01) were affected by the interaction of iron treatment and day. Data were subjected to ANOVA for repeated measures with a model that included iron treatment, diet, day, and all two- and three-way interactions as possible sources of variation. Within day post weaning, columns with different superscripts (^{a,b}) differ.



pigs receiving 200 mg at birth the least (70.0 ng/mL); pigs receiving 100 mg at birth and weaning had an intermediate concentration (79.8 ng/mL) not different from either of the other two groups. There was no effect of diet (*P* = .11) on hepcidin concentrations.

Iron treatment by diet by day (*P* = .19), and iron treatment by day (*P* = .43) did not affect concentrations of hepcidin in serum. There were tendencies, however, for hepcidin concentrations to be affected by iron treatment by diet (*P* = .06; Figure 4) and diet by day (*P* = .07; Figure 5). Hepcidin concentrations tended to be greater (*P* = .06) in control pigs receiving 100 mg iron at both birth and 14 days of age, compared to similarly treated copper-supplemented pigs (Figure 4). This dietary relationship did not exist for pigs treated with 200 mg of iron at birth (*P* = .99) or with 100 mg at both birth and weaning (*P* = .99). Hepcidin concentrations were similar on day 0 (*P* = .99) and day 7 (*P* = .99) post weaning between diets but tended to be greater (*P* = .07) on day 49 post weaning in control compared to copper-supplemented pigs (Figure 5).

BW and growth performance

There were no effects of treatment by diet on BW at weaning (day 0) or day 7, 21, or 49 post weaning (Table 3). Body weights at weaning were affected by iron treatment (*P* = .01). The mean (SE) BW of pigs that received 100 mg iron doses at birth and at day 14 of age (7.75 [0.53] kg) were greater (*P* = .01) than BW of pigs that received 100 mg iron doses at both birth and at weaning (7.29 [0.53] kg), with pigs that received 200 mg iron at birth having an intermediate value (7.47 [0.7] kg) that did not differ from the other two groups. In contrast, BW at days 7, 21, and 49 were not affected by iron treatment (Table 3). Diet affected BW at day 7 only with copper-supplemented pigs being heavier (*P* = .03) than their control counterparts.

Growth performance measures including ADG, ADFI, and G:F were not affected by iron treatment by diet for the periods from day 0 to 7, day 8 to 21, day 22 to 49, or day 0 to 49. Table 3 summarizes growth performance in nursery pigs as affected by the main effects of treatment and diet. Growth performance measures were similar among pigs receiving various iron treatment regimens for each period and the overall trial. Average daily gain was affected by diet for the period from weaning to day 7 only, with pigs consuming the copper-supplemented diet gaining faster (*P* = .03) than controls (139.4 [6.3] g/d versus 118.2 [6.3] g/d, respectively). All other growth performance measures were not affected by diet (Table 3).

Discussion

Iron is a critical component of hemoglobin, a protein molecule that allows red blood cells to carry oxygen from the lungs to bodily tissues and return carbon dioxide from tissues to the lungs. Anemia occurs when iron levels in the body are inadequate to maintain normal circulating concentrations of hemoglobin. Thus, the hemoglobin concentration in blood is a reliable indicator of iron status in swine.[21] Pigs with hemoglobin concentrations less than 9.0 g/dL are anemic and those with hemoglobin levels above 9.0 g/dL, but less than 11.0 g/dL, are iron-deficient.[4,22] In the current investigation, three different strategies for increasing blood iron concentrations in young pigs were compared in terms of hematology, hepcidin concentrations, and nursery growth performance.

Pigs receiving 100 mg iron injections at birth and weaning (after blood samples were collected) displayed a mean hemoglobin concentration (approximately 10 g/dL) consistent with iron deficiency. In contrast, pigs receiving 200 mg of iron at birth or 100 mg at both birth and day 14 of age, had sufficient iron stores available for hemoglobin synthesis. Similar to these results, Williams et al[2] reported that pigs administered 100 mg of gleptoferron 3 days after farrowing had mean hemoglobin concentrations at 21 days of age indicative of iron deficiency (approximately 9.3 g/dL). In that experiment, pigs receiving 150 or 200 mg of iron at 3 days of age or 200 mg of iron at both 3 and 11 days of age had weaning hemoglobin concentrations of 11.3, 12.0, and 12.8 g/dL, respectively. Chevalier et al[3] reported that pigs receiving 200 or 300 mg of iron at birth had normal levels of hemoglobin at weaning, but pigs that were injected with 100 mg of iron had mean hemoglobin concentrations indicative of anemia as early as 14 days of age.

Other hematological measures at approximately 22 days of age (weaning) in pigs receiving 100 mg iron at birth and at weaning in the current study, were also consistent with iron deficiency. Consonant with a previous report,[7] decreased hemoglobin concentrations were associated with decreased red blood cell concentration, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin concentrations, and increased red blood cell distribution width, a measure of variability in cellular size. The elevated levels of reticulocytes (immature red blood cells) seen in this study are consistent with increased production of these cells from bone marrow as a response to decreased iron levels.[23] Injection of an additional 100 mg of iron in these pigs restored hemoglobin, hematocrit, and mean corpuscular volume, but not mean corpuscular hemoglobin, red blood cell distribution width, and reticulocyte count to values similar to the other two treatment groups on day 7 post weaning. By day 49 post weaning, however, there were no differences in these measures among iron treatment regimens.

In general, the various hematological measures at weaning were similar for pigs receiving 200 mg of iron at birth and pigs receiving 100 mg of iron at both birth and day 14 of age. The responses observed here are consistent with that reported in a previous study during which hemoglobin concentrations at weaning were similar in pigs receiving 300 mg iron injections at birth or 200 mg iron at birth and 100 mg at 10 days of age, with animals in both treatment groups having greater hemoglobin levels than pigs receiving only 200 mg of iron at birth.[24]

Hepcidin, a protein hormone secreted by the liver, tightly controls iron availability in the body. In response to iron loading, hepatocytes release hepcidin. This hormone negatively affects the efflux of iron from duodenal enterocytes, and the release of iron from hepatocytes and macrophages. Collectively, these mechanisms prevent iron toxicity. In contrast, hepcidin expression is down regulated during iron deficiency, increasing iron availability. By controlling iron homeostasis, hepcidin strongly influences erythropoiesis.[18]

Lipiński et al[19] reported that administration of 200 mg of iron to neonatal pigs caused protracted increases in circulating concentrations of hepcidin, and elevated concentrations were still evident until at least 21 days of age. Starzyński et al[20] prevented iron deficiency anemia without affecting hepcidin concentrations by injecting pigs at 3 and 14 days of age with reduced amounts of iron dextran (37.5 mg/kg body weight). In the current experiment, hepcidin

Figure 2: Hematology characteristics of pigs receiving 200 mg of iron at birth, 100 mg iron at both birth and day 14 of age, or 100 mg iron at both birth and weaning (22.4 days of age) by intramuscular injection and fed control or copper-supplemented diets (14 or 250 ppm copper, respectively). A) Red blood cells ($P = .06$), B) eosinophils ($P = .05$), C) platelet number ($P = .06$), and D) platelet volume ($P = .09$) were affected by an interaction between iron treatment and day. Data were subjected to ANOVA for repeated measures with a model that included iron treatment, diet, day, and all two- and three-way interactions as possible sources of variation. Within iron treatment, columns with different superscripts (^{a,b,c}) differ.

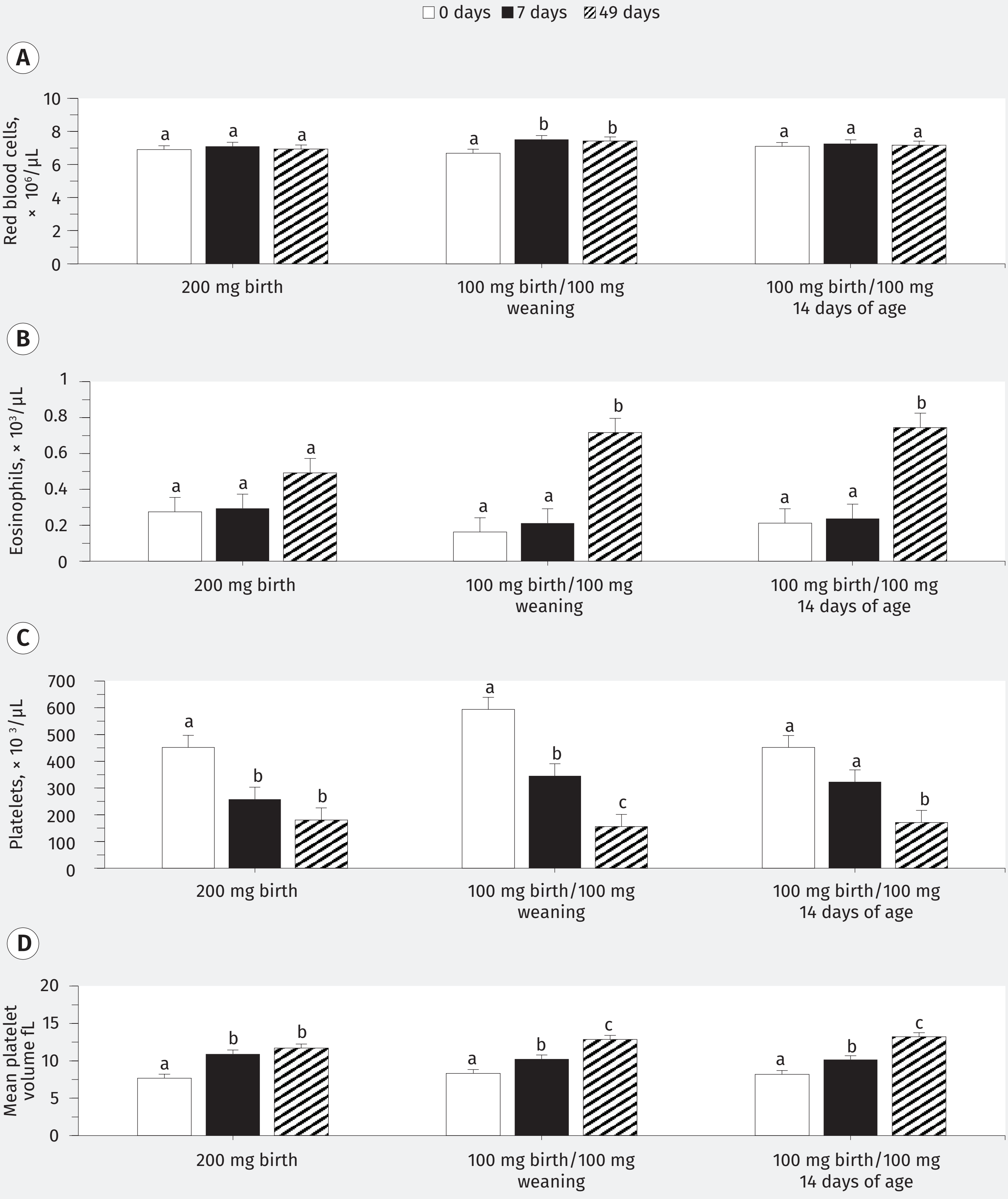
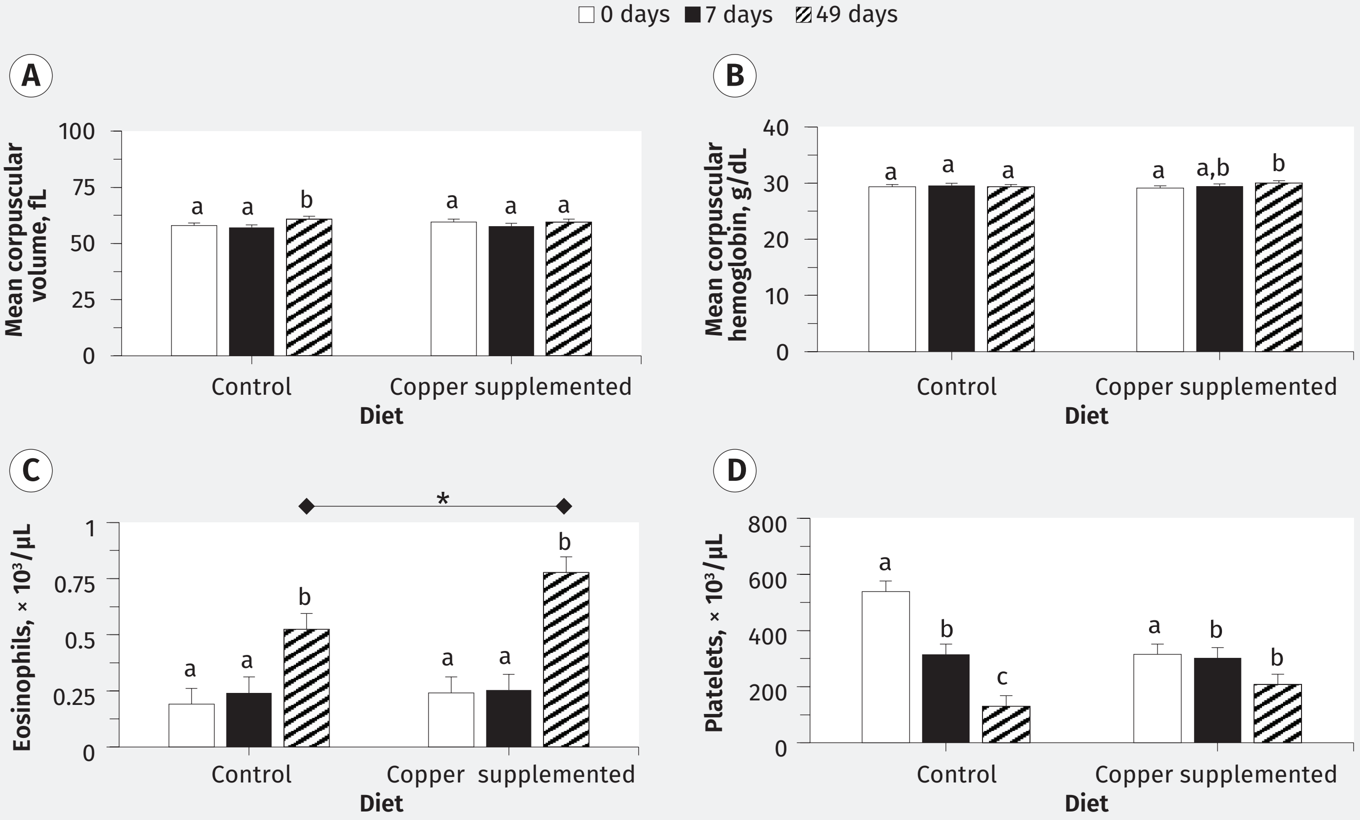


Figure 3: Hematology characteristics of pigs given 200 mg of iron at birth, 100 mg iron at both birth and day 14 of age, or 100 mg iron at both birth and weaning (22.4 days of age) by intramuscular injection and fed control or copper-supplemented diets (14 or 250 ppm copper, respectively). A) Mean corpuscular volume ($P = .06$), B) mean corpuscular hemoglobin ($P = .08$), C) eosinophil concentration ($P = .06$), and D) platelet number ($P = .03$) were affected by an interaction between diet and day. Data were subjected to ANOVA for repeated measures with a model that included iron treatment, diet, day, and all two- and three-way interactions as possible sources of variation. Within diet, columns with different superscripts (^{a,b,c}) differ. For eosinophils, concentrations tended to be greater ($P = .06$) on day 49 in copper-supplemented pigs versus controls (indicated with horizontal bar and *).



concentrations increased robustly with time post weaning. The values on day 49 post weaning, however, were undoubtedly influenced by consumption of dietary iron, in addition to the effects of the various iron injection regimens. Although there was no significant interaction of iron treatment and day post weaning across time points, hepcidin was greatest in the pigs that received 100 mg of iron at both birth and 14 days of age, and least in pigs receiving 200 mg of iron at birth only. In pigs receiving 100 mg iron at both birth and 14 d of age, hepcidin concentrations were greater in control versus copper-supplemented individuals. Additionally across iron treatments, hepcidin concentrations were greater in control versus copper-supplemented pigs on day 49 post weaning. To our knowledge, this is the first report of the effects of pharmacological levels of dietary copper on hepcidin concentrations in pigs. Dietary supplementation with copper has been demonstrated to decrease iron absorption.[14] Perhaps hepcidin concentrations decreased in copper-supplemented pigs as a mechanism to increase iron availability. Our finding that reticulocyte numbers were increased in pigs fed the copper-supplemented diet provides hematological support for this concept.

The transfer of weaned pigs to new surroundings in the nursery undoubtedly increased the antigenic load as evidenced by increases in indicators of both innate and acquired immunity. These temporal changes in white blood cell counts and the concentrations of neutrophils, monocytes, eosinophils, basophils, and lymphocytes are consistent with previous reports in the literature.[3,25-27] Moreover, decreases in platelet concentrations in pigs during the nursery phase of production have been previously shown.[7,27]

Numerous studies have demonstrated positive growth responses in nursery pigs provided concentrations of dietary copper in excess of nutritional requirements.[11-13] Consistent with previous reports, during the first week post weaning in this experiment, pigs fed the copper-supplemented diet exhibited greater weight gain and tendencies for greater feed intake and feed conversion efficiency compared to control pigs. In a previous study, ADG, ADFI, and G:F were enhanced by dietary copper in pigs that received 100 mg iron dextran at both birth and weaning but not in pigs receiving 100 mg iron at birth only, suggesting that an adequate iron status is requisite for copper to enhance growth performance in nursery pigs.[27] However, no measure of growth performance was influenced by the interaction of iron treatment regimen and diet in the current investigation. Thus, it appears that all 3 iron treatment regimens employed in

Figure 4: Hepcidin concentrations in pigs receiving 200 mg of iron at birth, 100 mg iron at both birth and day 14 of age, or 100 mg iron at both birth and weaning (22.4 days of age) by intramuscular injection and fed control or copper-supplemented diets (14 or 250 ppm copper, respectively). Blood was sampled on days 0, 7, and 49 post weaning. Hepcidin tended to be greater in control compared to copper-supplemented only in pigs that received 100 mg of iron at both birth and 14 days of age (*P* = .06; *). This dietary relationship did not exist in the other two groups. Data were subjected to ANOVA for repeated measures with a model that included iron treatment, diet, day, and all two- and three-way interactions as possible sources of variation.

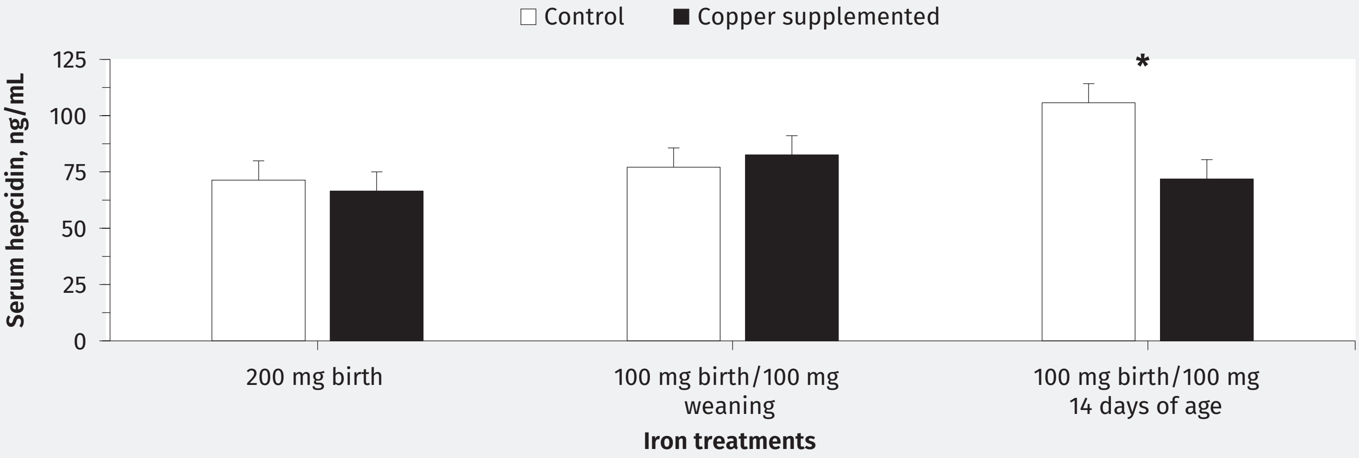
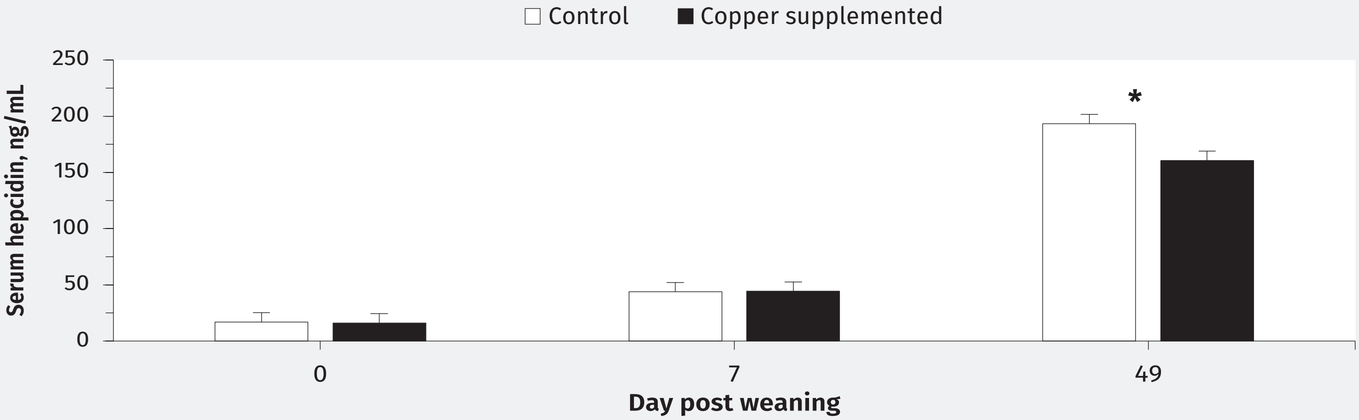


Figure 5: Hepcidin concentrations in pigs receiving 200 mg of iron at birth, 100 mg iron at both birth and day 14 of age, or 100 mg iron at both birth and weaning (22.4 days of age) by intramuscular injection and fed control or copper-supplemented diets (14 or 250 ppm copper, respectively). Blood was sampled on days 0, 7, and 49 post weaning. Data were subjected to ANOVA for repeated measures with a model that included iron treatment, diet, day, and all two- and three-way interactions as possible sources of variation. Concentrations of hepcidin tended to be affected (*P* = .07) by an interaction of diet and day post weaning with concentrations between diets tending to be different (*P* = .07; *) on day 49 only.



this study resulted in an iron status that allowed the weaned pigs to respond similarly to the supplemented copper.

Chevalier et al[3] administered increasing levels of iron at birth (0, 50, 100, 200, or 300 mg) and reported that pig weaning weights (day 22 of age) increased in both linear and quadratic fashions. Similarly, Williams et al[2] demonstrated that increasing amounts of iron (0, 50, 100, 150, and 200 mg) injected at day 3 of age resulted in linear and quadratic increases in ADG from day 3 to day 21 of age (weaning), with no increase in the response for doses greater than 100 mg. In the current study, pigs that received 100 mg of iron at both birth and day 14 of age had weaning BW that were greater than pigs receiving 100 mg of iron at both birth and weaning. Pigs that received 200 mg iron only at birth had weaning BW that were intermediate and not statistically different from the other two groups. Consistent with this finding, pigs receiving injections of 200 mg iron at both day 3 of age and 7 days prior to weaning at 28 days of age, had increased preweaning growth rates compared to pigs receiving 200 mg iron at birth only.[28] In contrast, preweaning ADG was not affected by an additional injection of 200 mg of iron 14 days prior to weaning at 34 days of age[29] or 100 mg of iron 10 days before weaning at 21 days of age.[2] Growth prior to weaning at approximately 17 days of age was similar among pigs treated with single doses of 200 or 300 mg of iron at birth or a 200 mg dose at birth followed by a 100 mg dose 10 days later.[24]

Table 3: Body weights and growth performance of pigs receiving different iron dextran treatment regimens and control (14 ppm) or copper-supplemented (250 ppm) nursery diets for 49 days

	Iron Treatment*											
	1			2			3			*P*†		
	Control (n = 4)	Copper (n = 4)	SE	Control (n = 4)	Copper (n = 4)	SE	Control (n = 4)	Copper (n = 4)	SE	Treatment	Diet	Treatment × Diet
Body weights, kg												
Weaning, D 0	7.49	7.46	0.54	7.19	7.39	0.54	7.67	7.83	0.54	.01	.37	.64
D 7	8.29	8.58	0.08	8.28	8.48	0.08	8.41	8.37	0.08	.75	.03	.12
D 21	12.63	12.84	0.44	12.31	13.25	0.45	13.10	13.18	0.45	.61	.26	.56
D 49	29.51	30.07	1.93	29.72	30.94	1.95	32.56	30.91	1.95	.60	.98	.74
D 0 to 7												
Gain, g/d	112.5	153.4	10.8	112.5	139.8	10.8	129.5	125.0	10.8	.80	.03	.13
Feed intake, g/d	247.7	288.6	14.5	244.3	256.8	14.5	262.5	262.5	14.5	.39	.11	.29
Gain:Feed, g/g	0.46	0.53	0.03	0.46	0.55	0.03	0.50	0.47	0.03	.75	.11	.16
D 8 to 21												
Gain, g/day	305.7	305.7	30.4	278.4	338.6	30.4	340.9	352.3	30.4	.30	.31	.54
Feed intake, g/d	504.5	504.5	48.4	534.1	560.2	48.4	559.1	558.0	48.4	.34	.79	.92
Gain:Feed, g/g	0.61	0.60	0.03	0.52	0.61	0.03	0.61	0.63	0.03	.15	.18	.19
D 22 to 49												
Gain, g/day	601.1	615.9	57.4	615.9	631.8	57.4	697.7	638.6	57.4	.57	.84	.76
Feed intake, g/d	1134.1	1172.7	114.9	1280.7	1227.2	114.9	1280.7	1354.5	114.9	.30	.73	.89
Gain:Feed, g/g	0.54	0.51	0.04	0.50	0.52	0.04	0.55	0.47	0.04	.81	.33	.31
Overall, D 0 to 49												
Gain, g/day	446.6	460.2	39.1	446.6	478.4	39.1	514.8	483.0	39.1	.49	.89	.71
Feed intake, g/d	828.4	855.7	80.0	903.2	897.7	80.0	929.5	970.5	80.0	.32	.72	.94
Gain:Feed, g/g	0.55	0.53	0.04	0.50	0.54	0.04	0.56	0.50	0.04	.71	.52	.17

* Treatment 1 = 200 mg iron at birth; Treatment 2 = 100 mg iron at birth and 100 mg iron at weaning (21 days of age); and Treatment 3 = 100 mg iron at birth and 100 mg iron at day 14 of age.

† Data were subjected to ANOVA. The model included iron treatment, diet, and the iron treatment by diet interaction as possible sources of variation.

In contrast to previous work[27] demonstrating a positive growth response to a second injection of 100 mg iron at weaning in pigs fed copper, post-weaning growth performance in the current investigation was similar among pigs receiving 200 mg of iron either as a single dose at birth or in equally divided doses given at birth and at day 14 of age or at weaning. Equivocal responses to a second iron injection before or at weaning on post-weaning growth performance have been reported. For example, pigs receiving injections of 200 mg iron at birth and 200 mg iron at 7 to 14 days prior to weaning had increased ADG compared to pigs receiving 200 mg iron at birth only.[28,29] In contrast, nursery growth performance after weaning was not dramatically affected by increasing the dosage of iron given at birth from 200 to 300 mg,[24,30] or by injecting 200 mg at birth and 100 to 200 mg at day 17 of age or at weaning.[24,31] Finally, increasing iron (0, 50, 100, or 200 mg) increased ADG and ADFI during the nursery phase of production with no effect of an additional injection of 100 mg at day 11 of age in pigs that received 200 mg at day 3 of age.[2] It is likely that any beneficial effects of additional iron treatment before or at weaning on growth performance is dependent on herd to herd factors such as iron status and diets.

Implications

Under the conditions of this study:

- Pigs receiving 100 mg of iron IM at birth were iron deficient at weaning.
- Additional 100 mg of iron given at 14 days of age increased weaning weights.
- Age-related increases in post-weaning hepcidin were dampened by copper supplementation.

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Conflict of interest

None reported.

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