Growth performance and hematology characteristics in pigs treated with iron at weaning as influenced by nursery diets supplemented with copper

Mark J. Estienne, PhD; Sherrie G. Clark-Deener, DVM, PhD; Kimberly A. Williams, BS

Summary

Objective: Determine the effects of dietary copper on growth in pigs given iron at weaning.

Materials and methods: Weanlings (n = 144) were allocated to a 2 × 2 × 2 factorial arrangement of treatments (6 pens/treatment, 3 pigs/pen). Factors were size (large or small), 100 mg intramuscular iron doses (birth or birth and weaning), and dietary copper (14.2 or 250 ppm). Average daily gain (ADG), feed intake (ADFI), and gain to feed ratio were determined for 49 days. Blood was sampled at weaning and days 7 and 49.

Results: Hemoglobin ($P < .001$) and hematocrit ($P = .002$) at weaning were less in large pigs. Pigs receiving two doses of iron had greater hemoglobin ($P = .05$) and hematocrit ($P = .04$). Hemoglobin ($P = .03$) and hematocrit ($P = .03$) were greater in pigs fed the control diet. In large pigs only, body weights at day 49 were greater ($P = .05$) for individuals receiving two doses of iron. The interaction between number of iron doses and diet affected many growth measures including ADG ($P = .02$) and ADFI ($P = .04$) for the overall trial. In all cases, performance was greater in copper-fed pigs receiving two doses of iron.

Implications: At weaning, larger pigs had hematology characteristics consistent with a lower iron status. Iron treatment at weaning increased hemoglobin. Copper enhanced growth only if pigs received iron at weaning. In copper-fed pigs, hemoglobin was less, possibly indicating a negative effect on iron absorption.

Keywords: swine, nursery, iron, copper, hematology

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Resumen - Desempeño de crecimiento y características hematológicas en cerdos tratados con hierro al destete según la influencia de las dietas de destete suplementadas con cobre

Objetivo: Determinar los efectos del cobre en la dieta sobre el crecimiento en cerdos que reciben hierro al destete.

Materiales y métodos: Se asignaron cerdos destetados (n = 144) a una disposición factorial de tratamientos 2 × 2 × 2 (6 corrales/tratamiento, 3 cerdos/corral). Los factores fueron el tamaño (grande o pequeño), dosis de hierro intramuscular de 100 mg (nacimiento o nacimiento y destete) y cobre dietético (14.2 o 250 ppm). La ganancia diaria promedio (ADG), el consumo de alimento (ADFI) y la relación ganancia alimento se determinaron durante 49 días. Se tomaron muestras de sangre al destete y los días 7 y 49.

Resultados: La hemoglobina ($P < .001$) y el hematocrito ($P = .002$) al destete fueron menores en los cerdos grandes. Los cerdos que recibieron dos dosis de hierro tuvieron mayor hemoglobina ($P = .05$) y hematocrito ($P = .04$). La hemoglobina ($P = .03$) y el hematocrito ($P = .03$) fueron mayores en los cerdos alimentados con la dieta control. Solo en cerdos grandes, el peso corporal en el día 49 fue mayor ($P = .05$) para los individuos que recibieron dos dosis de hierro. La interacción entre el número de dosis de hierro y la dieta afectó muchas medidas de crecimiento, incluyendo ADG ($P = .02$) y ADFI ($P = .04$) para la prueba en general. En todos los casos, el desempeño fue mayor en los cerdos alimentados con cobre que recibieron dos dosis de hierro.

Implicaciones: Al destete, los cerdos más grandes tenían características hematológicas consistentes con un estado de hierro más bajo. El tratamiento con hierro al destete aumentó la hemoglobina. El cobre aumentó el crecimiento solo si los cerdos recibieron hierro al destete. En los cerdos alimentados con cobre, la hemoglobina fue menor, posiblemente indicando un efecto negativo sobre la absorción de hierro.

Résumé - Performances de croissance et caractéristiques hématologiques chez des porcs traités avec du fer au moment du sevrage telles qu'influencées par une diète en pouponnière supplémentée avec du cuivre

Objectif: Déterminer les effets du cuivre dans l'alimentation sur les performances de croissance de porcs ayant reçu du fer au moment du sevrage.

MJE, KAW: Tidewater Agricultural Research and Extension Center, College of Agriculture and Life Sciences, Virginia Tech, Suffolk, Virginia.

SGC-D: Large Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Virginia.

Corresponding author: Dr Mark J. Estienne, 6321 Holland Road, Suffolk, VA 23437; Tel: 757-807-6551; Email: mestienn@vt.edu.

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Matériels et méthodes: Des porcelets sevrés (n = 144) furent assignés à un arrangement factoriel 2 x 2 x 2 de traitements (6 enclos/traitement, 3 porcs/enclos). Les facteurs étaient la taille (large ou petit), doses de 100 mg de fer intramusculaire (naissance ou naissance et sevrage) et cuivre alimentaire (14.2 ou 250 ppm). Le gain moyen quotidien (ADG), la prise d'aliment (ADFI) et le ratio gain/aliment furent déterminés pour 49 jours. Des échantillons de sang furent prélevés au sevrage ainsi qu'aux jours 7 et 49.

Résultats: Les valeurs d'hémoglobine ($P < .001$) et d'hématocrite ($P = .002$) au moment du sevrage étaient moindres chez les porcs larges. Les porcs ayant reçu deux doses de fer avaient des valeurs plus élevées d'hémoglobine ($P = .05$) et d'hématocrite ($P = .04$). Les valeurs d'hémoglobine ($P = .03$) et d'hématocrite ($P = .03$) étaient plus élevées chez les porcs nourris avec la diète témoin. Seulement chez les porcs de la catégorie large a-t-on remarqué que le poids corporel au jour 49 était plus élevé ($P = .05$) chez les individus recevant deux doses de fer. L'interaction entre le nombre de doses de fer et la diète affecta plusieurs mesures de la croissance incluant l'ADG ($P = .02$) et l'ADFI ($P = .04$). Dans tous les cas, les performances de croissance de porcs nourris avec une diète contenant du cuivre étaient meilleures chez ceux ayant reçus deux doses de fer.

Implications: Au moment du sevrage, les porcs de la catégorie large avaient des caractéristiques hématologiques compatibles avec un niveau de fer inférieur. Un traitement au fer au moment du sevrage augmenta l'hémoglobine. Le cuivra augmenta la croissance seulement si les porcs recevaient du fer au moment du sevrage. Chez les porcs nourris avec du cuivre, l'hémoglobine était moindre, indiquant un effet négatif possible sur l'absorption du fer.

Copper is an essential trace mineral used for the synthesis of hemoglobin and several oxidative enzymes critical for normal metabolism. Although the dietary copper requirement for weaned pigs is 5 to 6 ppm,[1] diets supplemented with levels of copper in excess of requirements (100 to 250 ppm) enhance growth during the nursery phase of production.[2-5] Dietary copper at levels deficient or in excess of nutritional requirements, however, have negative effects on iron absorption from the gastrointestinal tract.[6,7] Recent research has demonstrated that fast-growing pigs of modern genotypes are often iron deficient or anemic at weaning, despite having received intramuscular (IM) iron during the first week of life.[8-10] Clinically, pigs are considered anemic if blood concentrations of hemoglobin are less than 9.0 g/dL, and iron deficient if hemoglobin levels are above 9.0 g/dL but less than 11.0 g/dL.[8,11] An additional iron treatment at weaning could be important, particularly for nursery pigs consuming diets supplemented with pharmacological levels of copper to enhance growth performance. Thus, the experiment reported herein was conducted to determine the effects of an additional 100 mg iron treatment at weaning on growth performance and hematology characteristics in nursery pigs fed a diet supplemented with 250 ppm copper.

Materials and methods

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

Study animals and housing

Yorkshire × Landrace sows (n = 18) farrowed 169 Duroc-sired piglets, of which 144 high-health pigs (n = 76 males and n = 68 females) were employed. Piglets (n = 25) were excluded from the experiment because of unusually heavy or light body weights, signs of being unthrifty, hernias, or leg problems. Within 24 hours after birth, piglets were ear notched for identification, weighed, needle teeth were resected, and tails docked. All pigs received an IM injection of 100 mg iron hydrogenated dextran (Iron-100; Durvet, Inc) in the neck muscle behind the ear using a 20-gauge, 1.27 cm-long needle and a disposable 3 cc syringe (Becton, Dickinson and Company). To simulate commercial procedures, a standard amount of iron was administered shortly after birth rather than amounts based on body weight.[1] Similar to our previous work,[10] the dosage of iron was chosen because 1) lower doses are less likely to be toxic and cause oxidative stress; 2) greater doses of iron increase liver hepcidin secretion, which perturbs systemic iron metabolism; and 3) the 100 mg dose soon after birth would likely increase the number of anemic pigs at weaning, allowing for the evaluation of how these pigs respond to dietary copper supplementation. Boar piglets were castrated at seven days of age using a sterile scalpel. All piglets had access to sow feeders but no access to creep feed during the suckling period. At weaning, pigs were moved to an environmentally controlled nursery facility. Each nursery pen measured $0.91 \times 1.22\ m^2$ over galvanized steel bar slats and contained a nipple drinker and a stainless-steel feeder with four feeding spaces.

Study design

At 21.8 (0.5) days of age (mean [SE]), pigs were weaned, vaccinated against porcine circovirus type 2 and *Mycoplasma hyopneumoniae* (Circumvent PCV-M G2; Merck Animal Health), weighed, and divided into equal groups of the largest and smallest pigs (8.72 [0.40] and 5.97 [0.40] kg, respectively). Six blocks of eight pens each were created by placing a total of 12 pigs of each size category in pens of three pigs each. Each pen had at least one barrow and one gilt and pigs from at least two different litters. The eight pens within a block were randomly allocated to a 2 × 2 × 2 factorial arrangement of treatments. The factors were: 1) size of pig (large or small); 2) number of 100 mg IM iron doses (one dose administered within 24 hours after birth or two doses [one administered within 24 hours after birth and the other at weaning]); and 3) level of dietary copper (14.2 [control] or 250 ppm). There were six replicate pens per treatment combination (total of 48 pens).

Experimental diets

Pigs were allowed ad libitum access to a three-phase feeding regimen with all diets meeting the requirements for the various nutrients[1] and copper adjusted to concentrations previously indicated. For each phase, a basal diet was first prepared, containing most of the corn and all the common ingredients for each experimental diet. Copper sulfate (Pestell Minerals and Ingredients) or an equal amount of ground corn was added to the basal diet to create the copper or control diets, respectively (Table 1).

Data and sample collection

Pigs were weighed at weaning (day 0) and at days 7, 21, and 49 post weaning. Average daily gain (ADG) was determined for 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, average daily feed intake (ADFI) and the gain to feed ratio (G:F) could be calculated. Feed remaining in feeders was removed with a vacuum (Shop-vac) and weighed.

A blood sample from the barrow weighing closest to the mean weight of pigs in each pen was collected at weaning (before the

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

Feed component, %	Dietary phase (days fed post weaning) 1 (0 - 7)	2 (8 – 21)	3 (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 the basal diet consisting of the major portion of the ground corn and all other common ingredients. The control diet contained 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.

second dose of iron was administered to the appropriate pigs), and at days 7 and 49 post weaning. The same pig was used for each collection. For sampling, 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. Hematology analyses were conducted using a Coulter Multisizer 3 cell counter (Beckman Coulter, Inc) by Animal Laboratory Services of the Virginia-Maryland College of Veterinary Medicine (Blacksburg, Virginia). The following hematological determinations were made: number of red blood cells, reticulocytes, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and platelets, percentage of reticulocytes, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, red blood cell distribution width, and mean platelet volume.

Statistical analysis

Data were subjected to ANOVA using the mixed models procedure of SAS (SAS Institute Inc). Body weights, ADG, ADFI, and G:F were analyzed using a model that included pig size, number of iron doses, diet, and all two- and three-way interactions as possible sources of variation. Block was included as a random variable and pen served as the experimental unit. A repeated measures model was used for analyzing hematological characteristics and included pig size, number of iron treatments, diet, day, and all two-, three-, and four-way interactions as possible sources of variation. Block was included as a random variable and 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. Differences in means were considered statistically significant at $P < .05$.

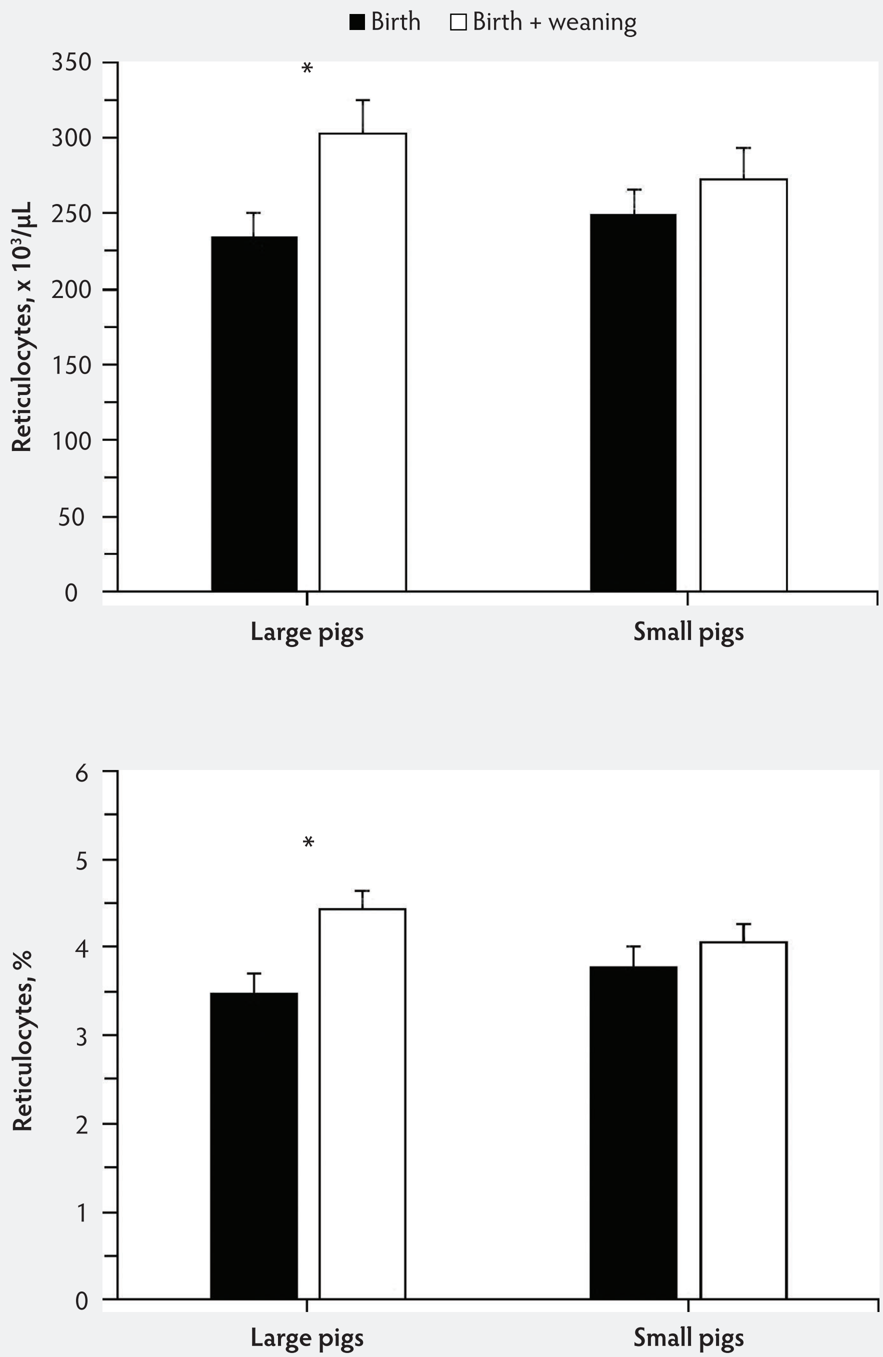
Results

There were no pig deaths or removals during the experiment.

Hematology characteristics

There were no three- or four-way interactions of main effects on hematology characteristics. The number and percentage of reticulocytes were affected ($P = .05$) by an interaction of pig size and the number of iron doses (Figure 1). In large pigs only,

Figure 1: Reticulocyte A) number and B) percentage (SE) in blood collected from large and small weaned pigs receiving one (birth) or two (birth and weaning) doses of 100 mg iron dextran by intramuscular injection. Data were subjected to ANOVA for repeated measures. Reticulocyte number and percentage were affected ($P = .05$) by the interaction of pig size and number of iron doses. The second dose of iron increased ($P = .05$; *) both values in large pigs but not small pigs.



reticulocyte number and percentage were greater ($P = .05$) for animals receiving two versus one iron dose.

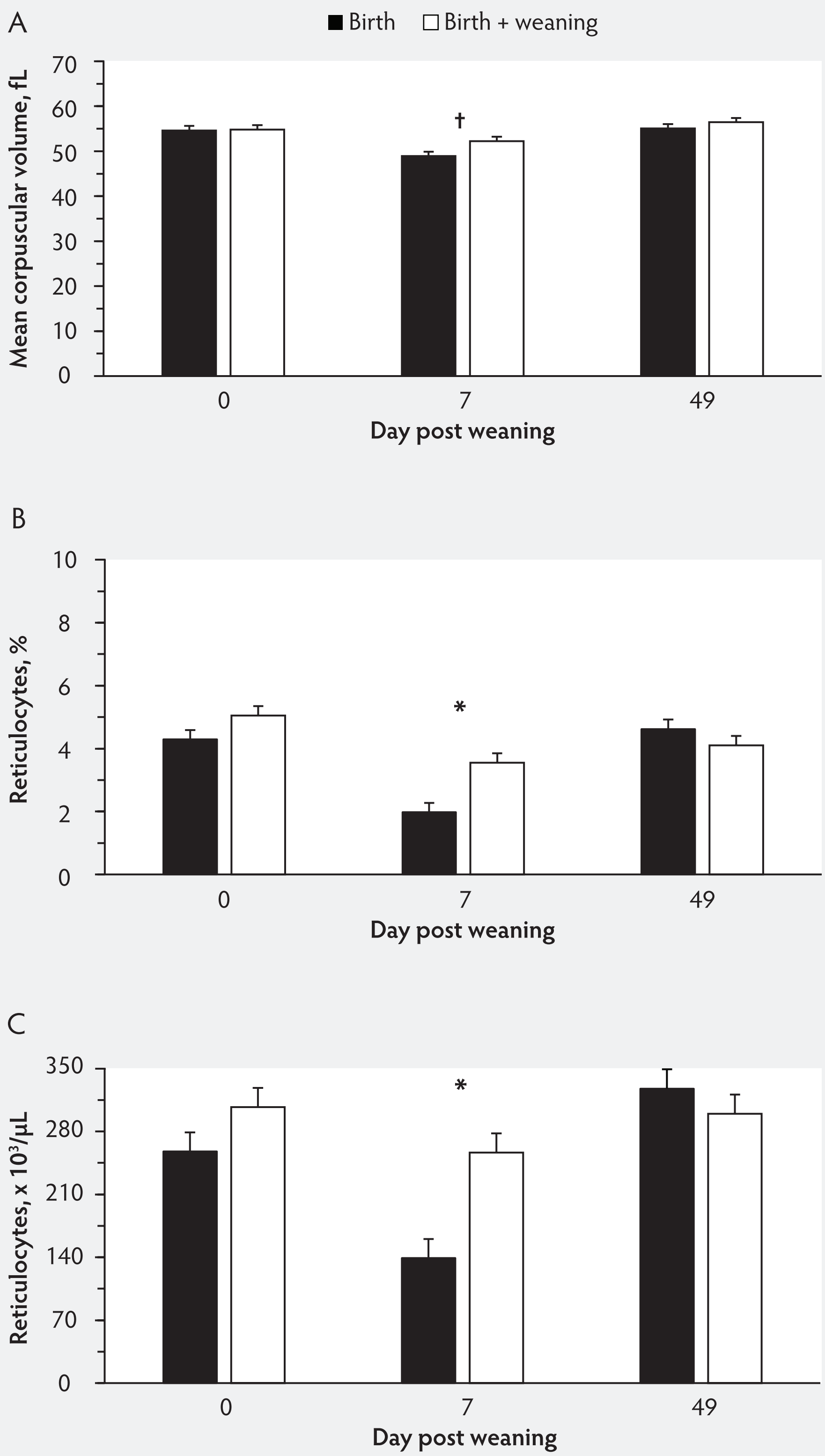
Mean corpuscular volume ($P = .01$) and reticulocyte percentage ($P = .001$) and number ($P = .001$) were affected by an interaction between number of iron doses and day post weaning (Figure 2). On day 7, but not on days 0 or 49, mean corpuscular volume ($P = .06$) and reticulocyte percentage ($P < .001$) and number ($P < .001$) were greater for pigs receiving two versus one iron dose.

There was an interaction of number of iron doses and diet for mean corpuscular hemoglobin ($P = .04$). Compared to pigs receiving iron only at birth, pigs receiving an additional iron dose at weaning had slightly greater mean corpuscular hemoglobin if fed the copper diet, but slightly decreased values if fed the control diet (Figure 3). Basophil concentration ($P = .05$; Figure 3) was also affected by an interaction of number of iron doses and diet. For pigs receiving only one dose of iron, basophil concentrations were less ($P = .04$) in animals fed copper. This effect of diet did not exist ($P = .98$) for pigs receiving iron at both birth and at weaning.

The interaction of pig size and day affected hemoglobin concentrations ($P < .001$), hematocrit ($P < .001$), mean corpuscular volume ($P < .001$), mean corpuscular hemoglobin ($P = .008$), red blood cell distribution width ($P = .001$), and reticulocyte percentage ($P = .02$) and number ($P = .02$; Figure 4). Hemoglobin concentrations and mean corpuscular volume on days 0 ($P < .001$ and $P < .001$, respectively) and 7 ($P = .02$ and $P < .001$, respectively), hematocrit on day 0 ($P = .002$), and mean corpuscular hemoglobin concentration on day 7 ($P = .008$) were less in large versus small pigs; there were no differences detected on day 49. In contrast, red blood cell distribution width was greater in the large pigs on both day 0 ($P = .001$) and 7 ($P = .002$). Reticulocyte percentage ($P = .09$) and number ($P = .04$) were greater for large versus small pigs on day 7, but not on the other days.

Table 2 contains hematology characteristics in nursery pigs as affected by the main effects of pig size, number of iron doses, diet, and day post weaning. Concentration of eosinophils ($P = .03$) were greater in the large versus small pigs. Hemoglobin ($P = .05$) and hematocrit ($P = .04$) were greater, and the number of platelets was less ($P = .05$) in pigs receiving iron doses at birth and at weaning

Figure 2: A) Mean corpuscular volume and reticulocyte B) percentage and C) number (SE) at day 0, 7, and 49 post weaning in pigs receiving one (birth) or two (birth and weaning) doses of 100 mg iron dextran by intramuscular injection. Data were subjected to ANOVA for repeated measures. Mean corpuscular volume (P = .01) and reticulocyte percentage (P < .001) and number (P < .001) were affected by an interaction between number of iron doses and day post weaning. Values were greater (P = .06, †; P < .001, *) on day 7 post weaning in pigs receiving two doses of iron.



compared to at birth only. Hemoglobin (P = .03), hematocrit (P = .03), and mean corpuscular volume (P = .04) were greater in pigs fed the control versus copper-supplemented diet. Red (P < .001) and white (P = .006) blood cell numbers, and mean platelet volume (P < .001) increased from day 0 to day 7, and then remained similar until day 49. Concentrations of lymphocytes (P < .001), monocytes (P < .001), basophils (P < .001), and platelets (P < .001) decreased from day 0 to day 7, and further decreased from day 7 to day 49.

Growth performance

There were no three-way interactions among pig size, number of iron doses, and diet for body weights at weaning or days 7, 21, or 49 post weaning. Day 49 body weights were affected by interactions of size of pig and number of iron doses (P = .05; Figure 5), and number of iron doses and diet (P = .04; Figure 6). In large pigs only, body weight was greater (P = .05) for individuals receiving two versus one dose of iron (Figure 5). Body weight was greater (P = .04) for copper-fed pigs that received two versus one dose of iron, however, body weights were not affected (P = .99) by the number of iron doses in control-fed pigs (Figure 6).

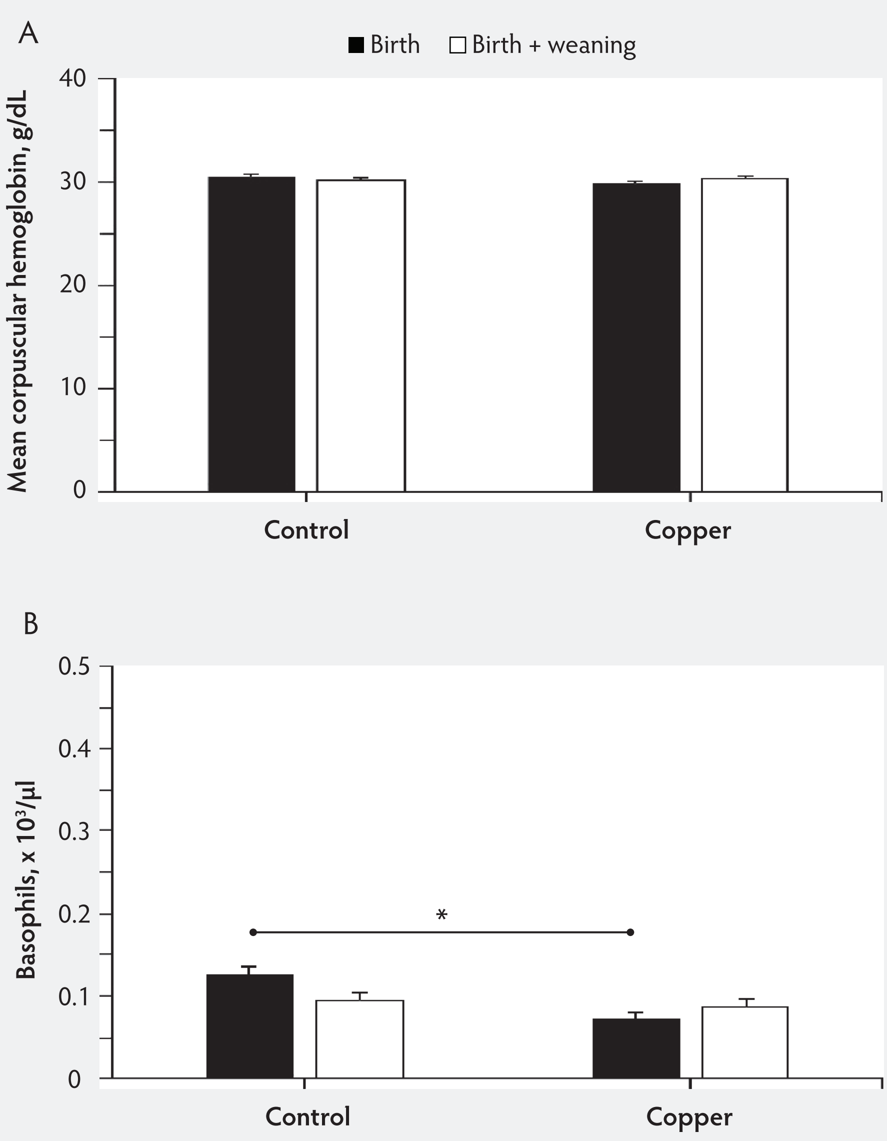
The interaction between number of iron doses and diet affected ADG and G:F from day 0 to 7 (P = .04 and P = .05, respectively) and day 8 to 21 (P = .009 and P = .01, respectively), ADFI from day 22 to 49 (P = .03), and ADG (P = .02) and ADFI (P = .04) for day 0 to 49 (Figures 7, 8, 9, and 10). In all cases, performance measures were greater in copper-fed pigs receiving two versus one dose of iron. In contrast, growth was unaffected by the number of iron doses in animals fed the control diet.

Table 3 summarizes body weights and growth performance in nursery pigs as affected by the main effects of size of pig, number of iron doses, and diet. Large pigs weighed more than small pigs on days 0, 7, and 21 of the experiment (P < .001). From day 0 (weaning) to 7, size of pig did not affect ADG (P = .50), ADFI (P = .18), or G:F (P = .18). For days 8 to 21 and 22 to 49, ADG and ADFI were greater in the large versus small pigs (P < .001 and P = .02, and P < .001 and P < .001, respectively). The G:F was also greater for large pigs from day 8 to 21 (P < .001) but not from day 22 to 49 (P = .41). For the overall trial (day 0 to 49 post weaning), ADG and ADFI were greater ($P < .001$) in large pigs, and G:F was similar ($P = .34$) for the different sized animals. Body weights were greater at day 21 ($P < .001$) in pigs receiving two versus one dose of iron.

Discussion

Hemoglobin is a protein molecule that allows red blood cells to carry oxygen from the lungs to bodily tissues and return carbon dioxide from tissues back to the lungs. Iron is a critical constituent of hemoglobin and iron deficiency anemia occurs if iron levels in the body are inadequate to maintain normal concentrations of hemoglobin in the blood. Clinically, pigs are considered anemic if blood concentrations of hemoglobin are less than 9.0 g/dL, and iron deficient if hemoglobin levels are above 9.0 g/dL but less than 11.0 g/dL.[8,11] To prevent iron deficiency anemia, pigs reared in confinement operations typically receive IM treatment with iron, usually in the form of iron dextran, within a few days after birth. The exact timing, dosage, and number of injections of iron dextran, however, varies widely among commercial pig farms.[12] Nevertheless, several research groups reported that a significant number of pigs, particularly the fastest growing animals within a litter, were iron deficient or anemic at weaning, despite receiving treatment with iron early in life,[8-10] and pigs that are anemic at weaning display poorer growth in the nursery compared with non-anemic pigs.[13] The results of the current experiment, when 100 mg iron was administered at birth, are consistent with those previous studies that demonstrated an increased risk of anemia at weaning in larger pigs. Indeed, small pigs weaned in the present study had greater hemoglobin concentrations, hematocrit, and mean corpuscular volume compared to large pigs. Mean corpuscular hemoglobin was also greater in small pigs on day 7 post weaning. In contrast, red blood cell distribution width, a measure of variability in the size of cells that increases in anemic individuals, was greater at weaning in the large versus small pigs. By the end of the 49-day trial there were no differences between size groups for hemoglobin, hematocrit, mean corpuscular volume, or red blood cell distribution width. In the current experiment, a standard dose of iron was employed and our finding that 100 mg iron was sufficient to prevent anemia in smaller but not larger pigs, suggests that body weight should be considered when iron is administered to newborns.

Figure 3: A) Mean corpuscular hemoglobin and B) number of basophils (SE) in control- or copper-fed pigs receiving one (birth) or two (birth and weaning) doses of 100 mg iron dextran by intramuscular injection. Data were subjected to ANOVA for repeated measures. Mean corpuscular hemoglobin ($P = .04$) and basophil concentration ($P = .05$) were affected by an interaction between number of iron doses and diet. For pigs receiving only one dose of iron, the copper ($P = .04$; *) but not control ($P = .98$) diet suppressed basophil concentrations.



Others have reported that eosinophil concentrations were less in anemic versus non-anemic pigs.[14] Interestingly, in the current study, eosinophil concentrations were greater in the pigs classified as large at weaning, despite the display of hematological data consistent with iron deficiency. Cases of concurrent iron deficiency anemia and eosinophilia have been reported in humans diagnosed with internal parasites,[15] and oral inoculation of pigs with infective ascaris eggs resulted in eosinophilia in the peripheral blood and a serum antibody response.[16] It is doubtful, however, that pigs in the current study had high numbers of internal parasites. The experiment was conducted in an intensively managed and highly sanitary university facility and sows were treated with 1.8% fenbendazole as per label (Safe-Guard; Merck Animal Health) before farrowing.

Hemoglobin concentrations, hematocrit, and mean corpuscular volume were greater in control pigs versus pigs fed a diet supplemented with copper. These findings are consistent with the hypothesis that pharmacological levels of dietary copper decrease iron absorption

Figure 4: Hematology characteristics (SE) in large and small pigs at 0, 7, and 49 days post weaning. Data were subjected to ANOVA for repeated measures. A) Hemoglobin (*P* < .001), B) hematocrit (*P* < .001), C) mean corpuscular volume (*P* < .001), D) mean corpuscular hemoglobin (*P* = .008), E) red blood cell distribution width (*P* = .001), F) reticulocyte percentage (*P* = .02), and G) reticulocyte number (*P* = .02) were affected by an interaction of pig size and day. For each hematology characteristic, bars for large and small pigs within day marked with an * differ (*P* < .05).

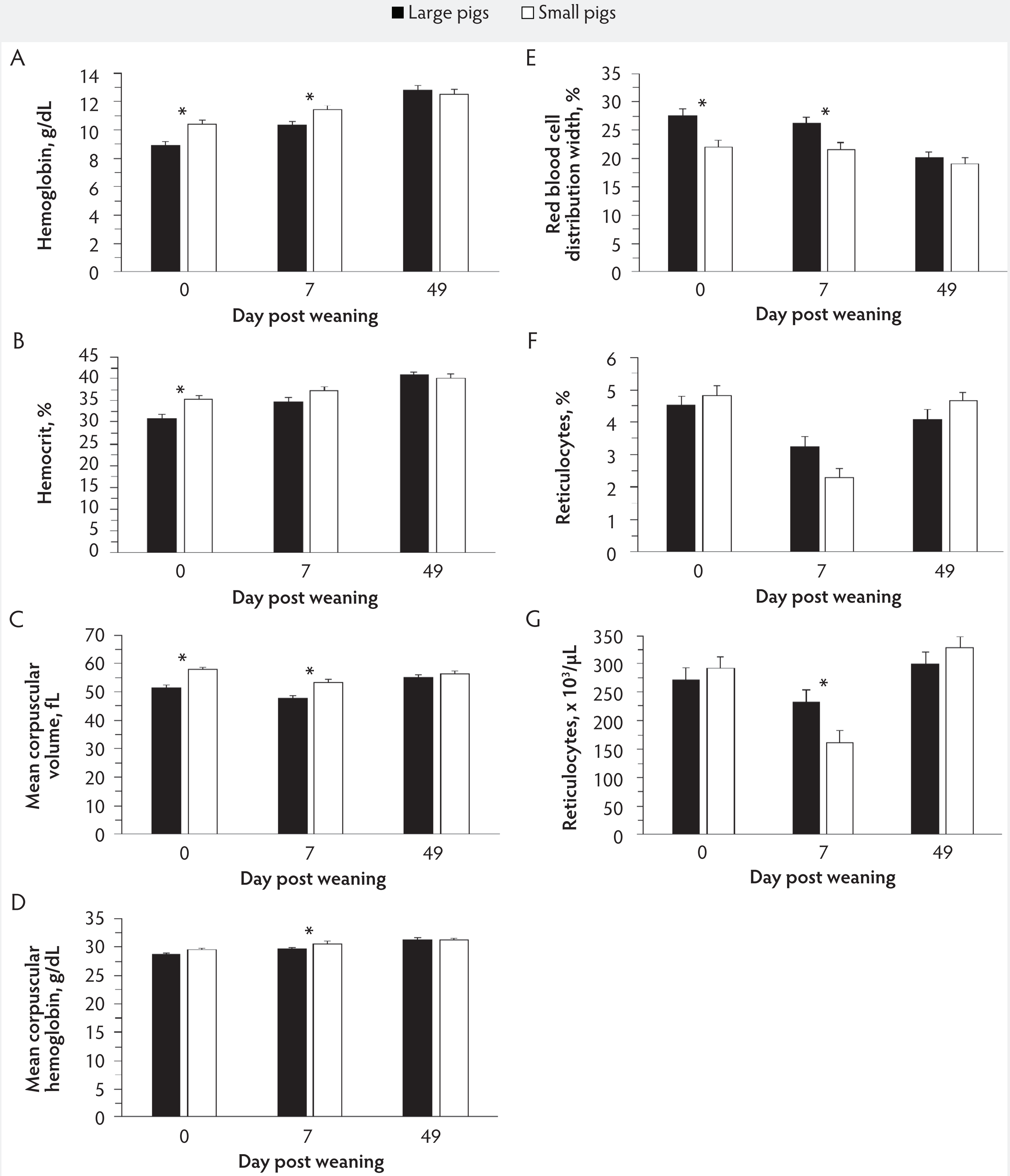


Table 2: Hematology characteristics of large and small nursery pigs treated intramuscularly with 100 mg iron dextran at birth or at birth and at weaning and fed control (14.2 ppm copper) or copper-supplemented (250 ppm) diets for 49 days

	Pig size				Iron doses (100 mg)				Diet				Day post weaning				
Hematological parameter	Large (n = 24)	Small (n = 24)	SE	P¶	Birth (n = 24)	Birth + weaning (n = 24)	SE	P¶	Control (n = 24)	Copper (n = 24)	SE	P¶	0 (n = 48)	7 (n = 48)	49 (n = 48)	SE	P¶
Red blood cells, $\times 10^6/\mu L$	6.90	6.74	0.09	.22	6.76	6.87	0.09	.41	6.86	6.78	0.09	.51	6.07[a]	7.13[b]	7.26[b]	0.09	< .001
Hemoglobin, g/dL*	10.67	11.44	0.24	.004	10.80	11.31	0.24	.05	11.36	10.76	0.24	.03	9.64[a]	10.86[b]	12.66[c]	0.23	< .001
Hematocrit, %*	35.49	37.55	0.72	.01	35.67	37.38	0.72	.04	37.44	35.60	0.72	.03	33.14[a]	35.95[b]	40.48[c]	0.71	< .001
Mean corpuscular volume, fL*†	51.48	55.88	0.86	< .001	52.87	54.50	0.86	.12	54.75	52.61	0.86	.04	54.72[a]	50.59[b]	55.74[a]	0.76	< .001
Mean corpuscular hemoglobin, g/dL*‡	29.92	30.46	0.24	.002	30.17	30.21	0.24	.81	30.28	30.10	0.24	.30	29.10[a]	30.18[b]	31.30[c]	0.25	< .001
Red blood cell distribution width, %*	24.65	20.90	1.04	< .001	23.55	21.99	1.04	.11	22.10	23.44	1.04	.16	24.88[a]	23.93[a]	19.51[b]	0.99	< .001
Reticulocytes, %*†§	3.95	3.91	0.18	.85	3.63	4.23	0.18	< .001	4.00	3.86	0.18	.43	4.67[a]	2.76[b]	4.36[a]	0.23	< .001
Reticulocytes, $\times 10^3/\mu L$*†§	268.8	260.5	13.2	.49	241.5	287.8	13.3	< .001	268.9	260.4	13.2	.48	282.4[a]	197.8[b]	313.7[a]	16.6	< .001
White blood cells, $\times 10^3/\mu L$	14.25	12.88	1.18	.41	12.75	14.39	1.18	.33	14.08	13.05	1.18	.53	9.95[a]	15.58[b]	15.17[b]	1.57	.006
Neutrophils, $\times 10^3/\mu L$	4.62	4.71	0.46	.84	4.25	5.08	0.46	.07	4.50	4.82	0.46	.48	4.30	5.33	4.36	0.51	.08
Lymphocytes, $\times 10^3/\mu L$	7.10	7.04	0.31	.88	7.33	6.81	0.31	.18	7.04	7.09	0.31	.91	4.78[a]	6.98[b]	9.44[c]	0.31	< .001
Monocytes, $\times 10^3/\mu L$	0.47	0.53	0.04	.11	0.50	0.51	0.04	.84	0.50	0.50	0.04	.95	0.26[a]	0.52[b]	0.73[c]	0.05	< .001
Eosinophils, $\times 10^3/\mu L$	0.51	0.37	0.06	.03	0.42	0.46	0.06	.52	0.42	0.46	0.06	.51	0.43	0.50	0.39	0.06	.12
Basophils, $\times 10^3/\mu L$‡	0.09	0.10	0.01	.48	0.10	0.09	0.01	.49	0.11	0.08	0.01	.01	0.04[a]	0.10[b]	0.14[c]	0.01	< .001
Platelets, $\times 10^3/\mu L$	416.8	412.8	30.0	.91	450.0	379.6	30.0	.05	382.6	447.0	30.0	.07	530.7[a]	405.0[b]	308.8[c]	28.9	< .001
Mean platelet volume, fL	9.65	9.71	0.51	.85	9.60	9.77	0.52	.61	9.53	9.84	0.52	.36	10.56[a]	9.25[b]	9.23[b]	0.30	< .001

* Affected by interaction of pig size and day ($P < .001$ for hemoglobin, hematocrit, mean corpuscular volume; $P = .001$ for red blood cell distribution width; $P = .008$ for mean corpuscular hemoglobin; and $P = .02$ for reticulocyte number and percentage).

† Affected by interaction of iron treatments and day ($P = .001$ for reticulocyte number and percentage; and, $P = .01$ for mean corpuscular volume).

‡ Affected by interaction of iron treatments and diet ($P = .04$ for mean corpuscular hemoglobin, and $P = .05$ for basophils).

§ Affected by interaction of pig size and iron treatments ($P = .05$ for reticulocyte number and percentage).

¶ Data were subjected to ANOVA for repeated measures. For the main effect of day, values with different superscripts (a,b,c) differ ($P < .05$).

in pigs.[6,7] Interestingly, basophil concentrations in pigs receiving iron at birth only were less in copper-fed individuals compared to controls. The biological significance of this finding, however, is unclear.

In the current experiment, hemoglobin concentrations, hematocrit, and reticulocyte number and percentage were affected by day of sampling and pig size. Hemoglobin and hematocrit were greater, and platelet counts were less, in pigs receiving an additional iron injection at weaning. These changes reflect a positive effect of iron therapy in individuals that may be anemic or iron deficient, and are consistent with previous reports in the literature.[17,18] For example, a second injection of 200 mg iron dextran at 20 days of age increased hemoglobin concentrations in pigs weaned and blood sampled at 34 days of age.[18] Our finding that platelet counts were less in pigs receiving an additional dose of iron at weaning are consistent with a previous study in which humans with iron deficiency anemia had greater platelet counts than those with adequate iron stores; oral iron supplementation decreased platelet counts in anemic individuals but not in those with normal iron levels.[19]

Figure 5: Body weights (SE) at day 49 post weaning in large and small pigs receiving one (birth) or two (birth and weaning) doses of 100 mg iron dextran by intramuscular injection. Data were subjected to ANOVA. Bars within pig size marked with an * differ ($P < .05$).

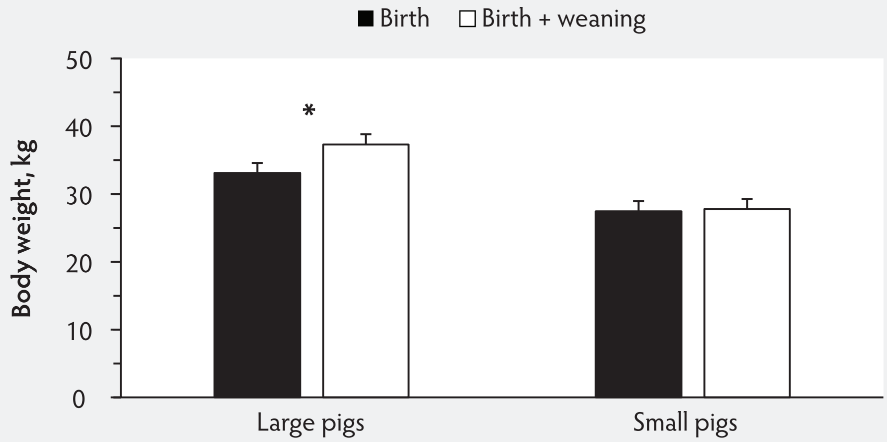
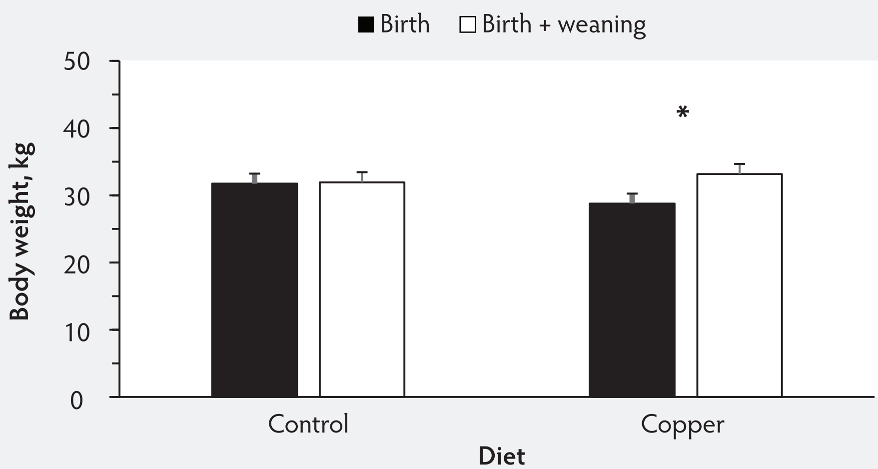


Figure 6: Body weights (SE) at day 49 post weaning in control- (14.2 ppm copper) and copper-fed (250 ppm) pigs receiving one (birth) or two (birth and weaning) doses of 100 mg iron dextran by intramuscular injection. Data were subjected to ANOVA. Bars within diet marked with an * differ ($P < .05$).

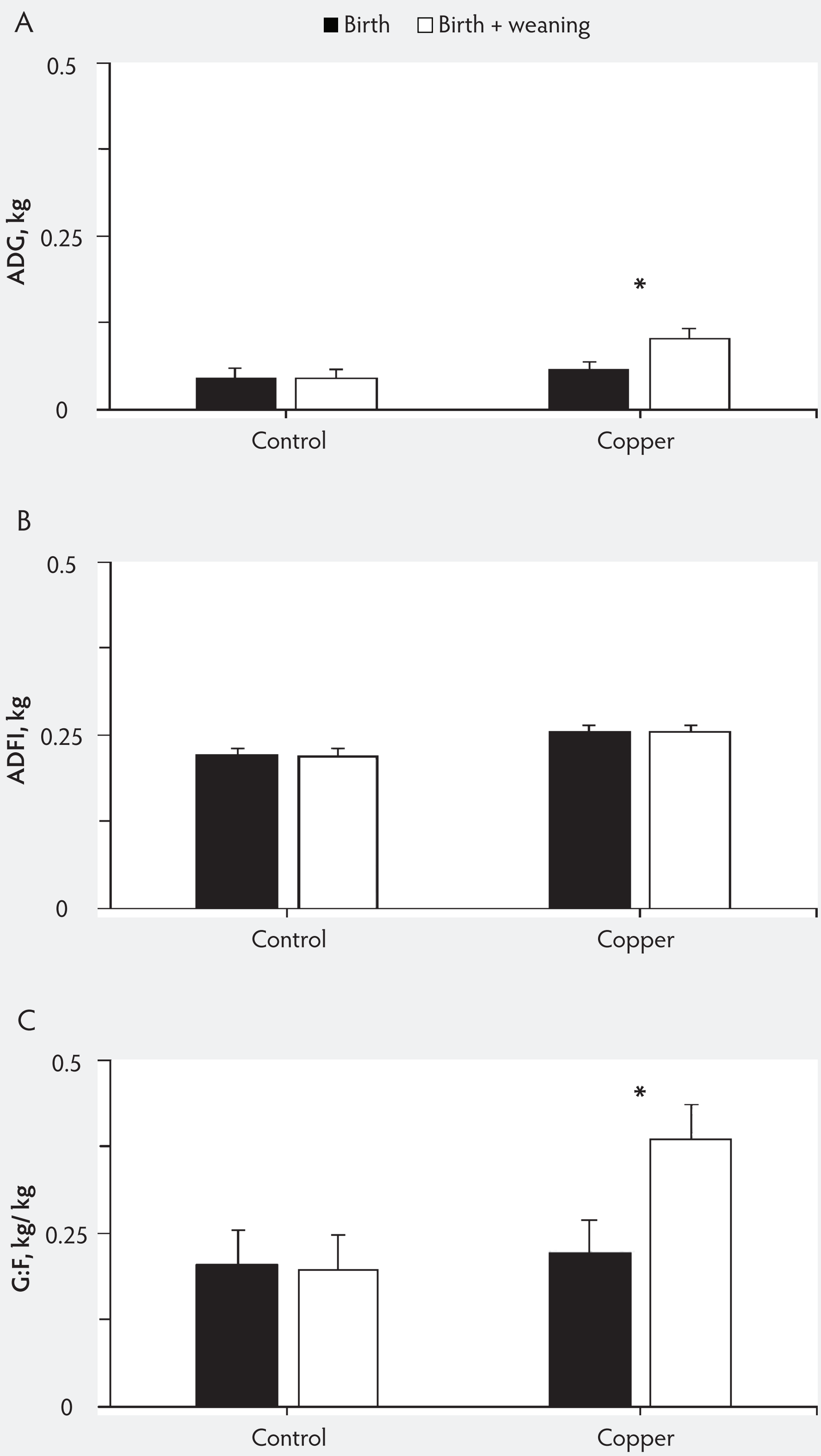


For the study reported here, pigs classified as large at weaning weighed approximately 2.8 kg more than pigs classified as small. The difference between size groups increased during the study and was approximately 7.6 kg at day 49 post weaning. That larger pigs at weaning maintain or expand a size advantage over small pigs at weaning has been previously reported.[10,20-23] Feed conversion efficiency is a function of body weight, and as a pig grows, it may become less efficient at converting feed into body weight gain,[24] which could explain our finding that small pigs displayed greater G:F from day 8 to 21 in the nursery than large pigs. In the current experiment, however, final body weight was impacted by an interaction between pig size and the number of iron doses. As mentioned above, hemoglobin levels were less in large versus small pigs at weaning. Perhaps an iron deficiency was mitigated by iron treatment at weaning, allowing the large pigs to achieve maximum size. Moreover, we cannot discount potential effects of dietary iron on improved growth responses.

Increased growth in nursery pigs provided pharmacological concentrations of dietary copper have been well-documented[2-5] and, consistent with previous reports, pigs fed the copper-supplemented diet in the current study exhibited greater weight gain, feed intake, and feed conversion efficiency than control pigs during the first week post weaning. More importantly, many aspects of growth performance were influenced by an interaction between the number of iron treatments and diet. Indeed, ADG (days 0 to 7, 8 to 21, and 0 to 49), ADFI (days 22 to 49 and 0 to 49) and G:F (days 0 to 7 and 8 to 21) were enhanced by dietary copper only if an additional 100 mg iron dose was administered at weaning. Based on these results, it appears that an adequate level of iron in the body is requisite for dietary copper to enhance growth performance in nursery pigs. As reported, iron injected at weaning did not enhance growth performance in pigs fed the control diet.

Studies in which additional iron was given by either increasing the dosage administered at birth or by administering an additional dose during the suckling period or at weaning, have yielded equivocal growth responses. Consistent with our results for the pigs receiving copper, 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.[18,25] In contrast, growth

Figure 7: A) ADG, B) ADFI, and C) G:F (SE) between day 0 and 7 post weaning in pigs that received one (birth) or two (birth and weaning) doses of 100 mg iron dextran and were fed a control diet (14.2 ppm copper) or diet supplemented with 250 ppm copper. Data were subjected to ANOVA. Average daily gain ($P = .04$) and G:F ($P = .05$) were affected by an interaction of diet and number of iron doses. In copper-fed pigs only, ADG and G:F were greater ($P < .05$; *) for individuals receiving two versus one dose of iron. ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.



performance was not affected or was only slightly influenced by increasing the dosage of iron given at birth from 200 to 300 mg,[26,27] or by injecting 200 mg at birth and 100 to 200 mg at 17 days of age or at weaning.[17,27]

In summary, hematological analyses conducted in this study reflect an increased risk of anemia at weaning in larger pigs. That 100 mg of iron given at birth was sufficient to prevent anemia in smaller but not larger pigs suggests that body weight should be considered when iron is administered to newborns. Based on hematological evidence, high levels of dietary copper appear to decrease iron absorption, and it appears that an adequate level of iron in the body is requisite for dietary copper to enhance growth performance in nursery pigs. These findings illustrate the complex relationship among trace minerals in swine and the need for further research in this area of nutrition.

Implications

Under the conditions of this study:

- Iron treatment at weaning increased hemoglobin levels.
- Copper enhanced nursery growth only if pigs received iron at weaning.
- Hemoglobin levels were less in copper-fed pigs compared to controls.

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Conflict of interest

None reported.

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Figure 8: A) ADG, B) ADFI, and C) G:F (SE) between day 8 and 21 post weaning in pigs that received one (birth) or two (birth and weaning) doses of 100 mg iron dextran and were fed a control diet (14.2 ppm copper) or diet supplemented with 250 ppm copper. Data were subjected to ANOVA. Average daily gain (*P* = .009) and G:F (*P* = .01) were affected by an interaction of diet and number of iron doses. For each performance measure, bars with different superscripts differ (*P* < .05). ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

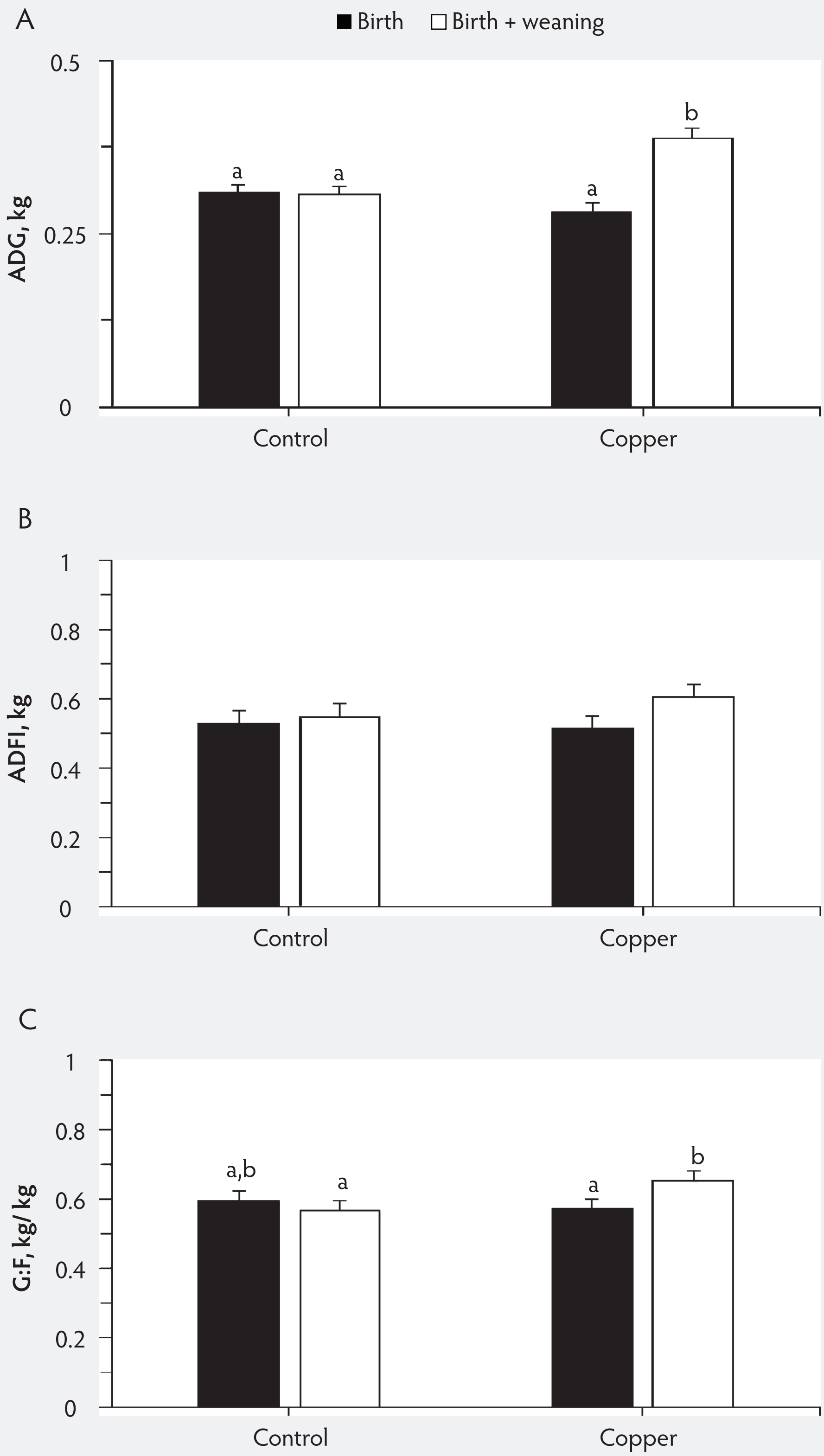

Figure 9: A) ADG, B) ADFI, and C) G:F (SE) between day 22 and 49 post weaning in pigs that received one (birth) or two (birth and weaning) doses of 100 mg iron dextran and were fed a control diet (14.2 ppm copper) or diet supplemented with 250 ppm copper. Data were subjected to ANOVA. Average daily feed intake (*P* = .03) was affected by an interaction of diet and number of iron doses, and in copper-fed pigs was greater (*P* = .03; *) for individuals receiving two versus one dose of iron. ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.

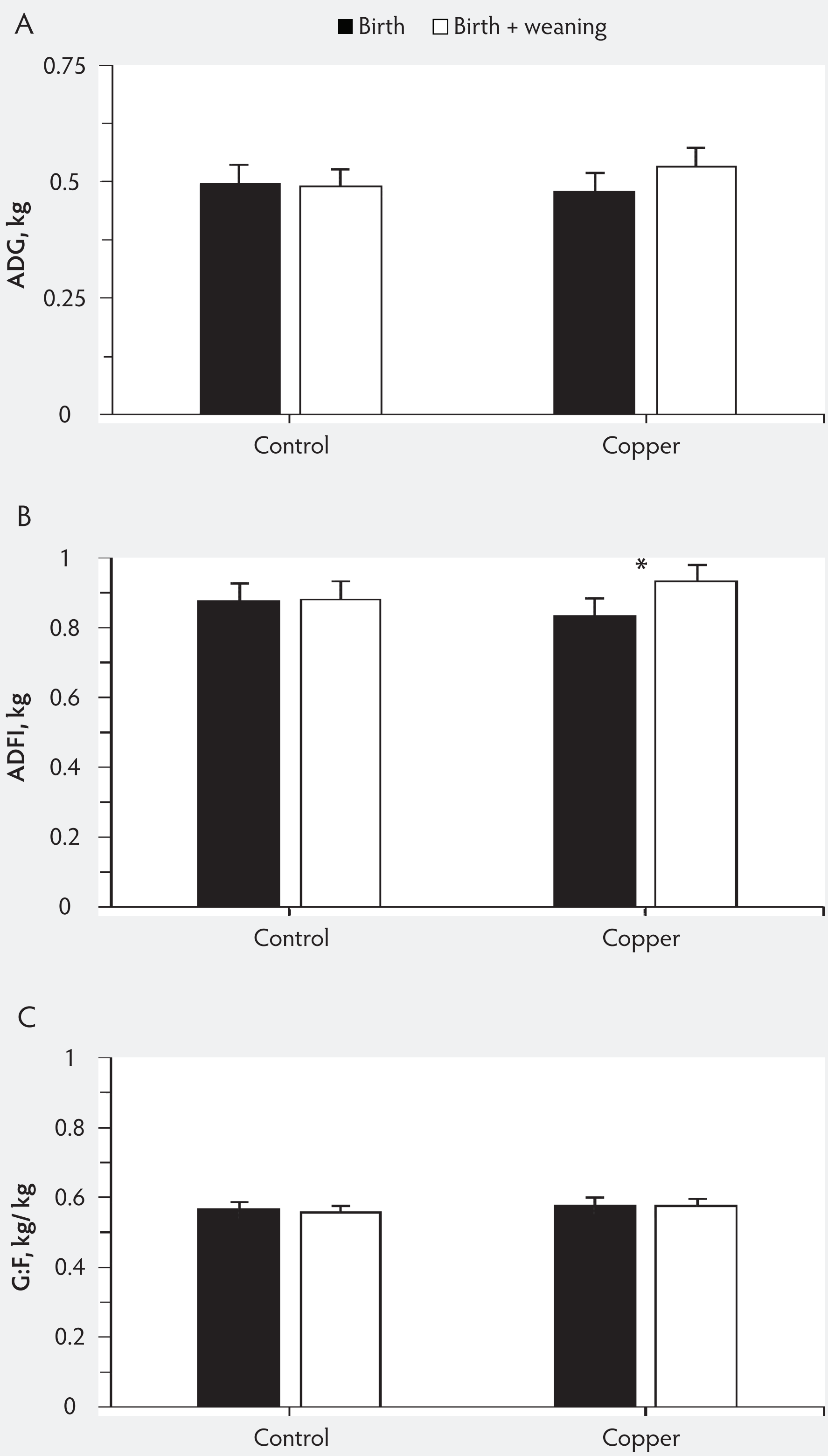

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Figure 10: A) ADG, B) ADFI, and C) G:F (SE) between weaning (day 0) and day 49 post weaning in pigs that received one (birth) or two (birth and weaning) doses of 100 mg iron dextran and were fed a control diet (14.2 ppm copper) or diet supplemented with 250 ppm copper. Data were subjected to ANOVA. Average daily gain ($P = .02$) and ADFI ($P = .01$) were affected by an interaction of diet and number of iron doses. Both performance measures were greater ($P < .05$; *) in copper-fed pigs receiving two versus one dose of iron.

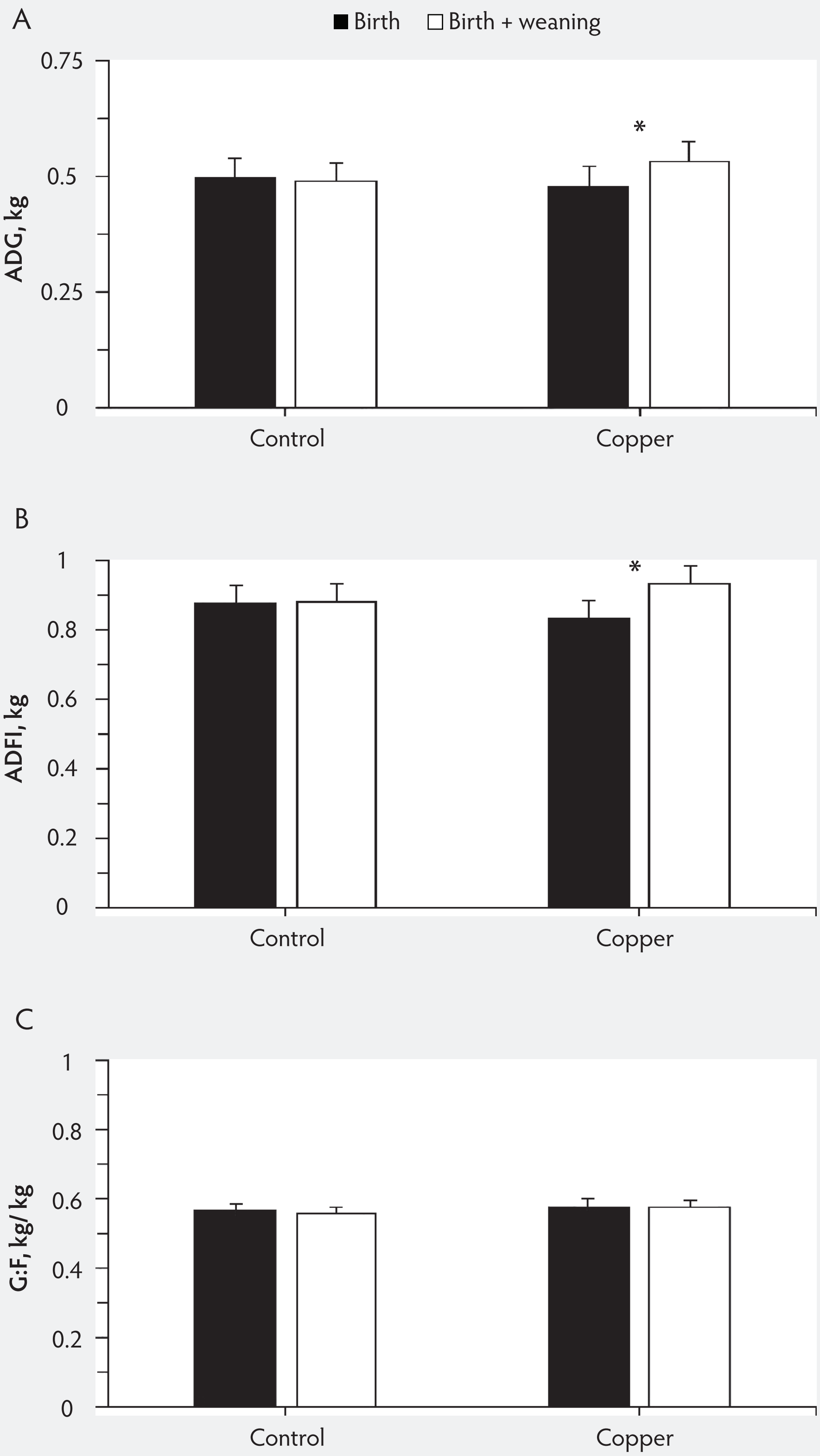

Table 3: Body weights and growth performance of large and small nursery pigs injected once or twice with iron dextran (100 mg) and fed control (14.2 ppm copper) or copper (250 ppm) supplemented diets for 49 days

	Pig size				Iron doses (100 mg)				Diet			
Items:	Large (n = 24)	Small (n = 24)	SE	$P^{§}$	Birth (n = 24)	Birth + Weaning (n = 24)	SE	$P^{§}$	Control (n = 24)	Copper (n = 24)	SE	$P^{§}$
Body weights, kg												
Day 0	8.72	5.97	0.40	< .001	7.35	7.34	0.40	.99	7.36	7.32	0.40	.82
Day 7	9.11	6.37	0.38	< .001	7.66	7.82	0.38	.48	7.68	7.80	0.38	.57
Day 21	14.05	9.90	0.56	< .001	11.29	12.66	0.56	.001	12.09	11.86	0.56	.64
Day 49*†	35.20	27.62	1.33	< .001	30.26	32.56	1.33	.02	31.83	30.99	1.33	.38
Day 0 to 7												
ADG, kg/d‡	0.06	0.07	0.01	.50	0.05	0.07	0.01	.05	0.05	0.08	0.01	.004
ADFI, kg/d	0.24	0.23	0.01	.18	0.24	0.24	0.01	.96	0.22	0.25	0.01	.006
G:F‡	0.22	0.28	0.04	.18	0.21	0.29	0.04	.06	0.20	0.30	0.04	.02
Day 8 to 21												
ADG, kg/d‡	0.37	0.28	0.02	< .001	0.29	0.35	0.02	.01	0.31	0.33	0.02	.18
ADFI, kg/d	0.58	0.52	0.03	.02	0.52	0.58	0.02	.03	0.54	0.56	0.02	.40
G:F‡	0.64	0.55	0.07	< .001	0.58	0.61	0.07	.24	0.58	0.61	0.07	.13
Day 22 to 49												
ADG, kg/d	0.75	0.66	0.02	< .001	0.70	0.71	0.02	.50	0.71	0.70	0.02	.97
ADFI, kg/d‡	1.31	1.12	0.03	< .001	1.19	1.25	0.03	.04	1.22	1.21	0.03	.64
G:F	0.58	0.59	0.01	.41	0.59	0.57	0.01	.10	0.58	0.59	0.01	.45
Day 0 to 49												
ADG, kg/d‡	0.54	0.46	0.02	< .001	0.49	0.51	0.02	.07	0.49	0.50	0.02	.35
ADFI, kg/d‡	0.94	0.82	0.02	< .001	0.85	0.90	0.02	.03	0.88	0.88	0.02	.87
G:F	0.57	0.56	0.02	.34	0.57	0.57	0.02	.55	0.56	0.58	0.02	.09

* Affected (P = .05) by interaction between size of pig and number of iron treatments.

† Affected (P = .04) by interaction of number of iron treatments and diet.

‡ Affected by interaction of number of iron treatments and diet (Day 0 to 7, P = .04 for ADG and P = .05 for G:F; Day 8 to 21, P = .009 for ADG and P = .01 for G:F; Day 22 to 49, P = .03 for ADFI; and Day 0 to 49, P = .02 for ADG and P = .04 for ADFI).

§ Data were subjected to ANOVA.

ADG = average daily gain; ADFI = average daily feed intake; G:F = gain to feed ratio.