Title: Enhancing growth performance in pigs by treating with iron at weaning and feeding antibiotic-free nursery diets containing elevated concentrations of copper or zinc **- NPB #17-052**

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Industry Summary:

Addition of copper sulfate or zinc oxide to nursery pig diets at levels in excess of dietary requirements was previously shown to enhance growth performance. In the new era of antimicrobial use restrictions, the proportion of farmers incorporating elevated concentrations of these trace minerals in nursery pig diets will likely increase. Both copper and zinc, however, have been reported to inhibit iron absorption and research suggests that high dietary concentrations of zinc oxide are associated with a greater risk of anemia in pigs after weaning. Iron deficiency anemia at weaning is an emerging issue, and it has been suggested that the traditional treatment of pigs early in life with 100 to 200 mg of iron may be insufficient to meet requirements of large, fast-growing individuals, and a second injection of iron may be indicated. The additional iron treatment could be particularly important for nursery pigs consuming diets with elevated levels of copper or zinc, especially during the early post-weaning period. The objectives of the research reported here were to determine the effects of iron injections at weaning on hematology characteristics and growth performance in nursery pigs fed antibiotic-free diets containing elevated levels of copper (Experiment 1) or zinc (Experiment 2). In each experiment, pigs received intramuscular injections of 100 mg iron dextran within 24 hours after birth. At weaning (21 days of age) pigs were classified as large or small and one-half of pigs of each size received a second injection of 100 mg iron. Pigs were placed three pigs/nursery pen, as per a 2 x 2 x 2 factorial arrangement of treatments, main factors being size (large vs. small), iron injections (one vs. two) and diet (control vs. 250 ppm copper [Experiment 1] or 2000 ppm zinc [Experiment 2]) (n = 6 pens/treatment combination in each experiment). Growth performance was measured, and hematology characteristics assessed (one pig/pen) at weaning (day 0) and days 7 and 49 post-weaning. Of 90 pigs blood sampled at weaning in Experiments 1 and 2, more ($P < 0.01$) large (24/45, 53.3%), compared to small (7/45, 15.6%) pigs were classified as anemic, having hemoglobin concentrations of less than 9 g/dL. For the pigs blood sampled, growth during the first seven days post-weaning was affected ($P = 0.04$) by an interaction between anemic status and iron treatment at weaning. The average daily gain (**ADG**) of anemic and non-anemic pigs that received iron treatment at weaning were similar ($P > 0.05$). In contrast, non-anemic pigs not receiving an iron treatment at weaning had greater ($P < 0.05$) ADG compared to their anemic counterparts. Over the course of the entire trial, ADG was not affected by anemic status, number of iron treatments, or the interaction between these factors. Consistent with the hypothesis that elevated levels of copper negatively impact iron absorption, in Experiment 1, hemoglobin concentrations ($P = 0.02$), and hematocrit ($P = 0.02$), were greater in pigs fed control diets (11.4 ± 0.3 g/dL and 37.5 ± 0.9%, respectively) compared to pigs fed diets containing 250 ppm copper (10.8 ± 0.3 g/dL and 35.7 ± 0.9%, respectively). From day 0 (weaning) until day 7 post-weaning, ADG and GF were affected ($P = 0.04$) by an interaction between treatment at weaning (in other words, iron or no iron) and diet (copper-supplemented or control). For pigs fed the copper diet, animals treated with iron at weaning had greater ($P < 0.05$) ADG and GF than animals not treated with iron. The additional iron treatment at weaning had no effect ($P > 0.05$) on ADG or GF in pigs consuming the control diet. From day 0 until day 49 post-weaning (entire trial), ADG ($P = 0.02$) and ADFI ($P = 0.04$) were affected by an interaction between iron treatment and diet. For pigs fed the copper diet, animals treated with iron at weaning had greater ($P < 0.05$) ADG and ADFI than animals not treated with iron. The additional iron treatment at weaning had no effect ($P > 0.05$) on ADG or ADFI in pigs consuming the control diet. From day 0 to 7 in Experiment 2, zinc-fed pigs had greater ($P < .01$) ADG and GF, but similar ($P = .14$) ADFI compared to controls. Over the course of the 49-day trial, pigs fed diets supplemented with zinc had greater ADFI ($P < .01$) but similar ADG ($P = .44$) and GF ($P = .23$) compared with controls. In contrast to Experiment 1, there was no interaction of the zinc diet and number of iron treatments. Research conducted by our team has demonstrated that: 1) Consistent with previous work at Virginia Tech and other institutions, a significant proportion of pigs are anemic at weaning, despite having received i.m. iron shortly after birth; 2) Within litters, the larger, faster growing individuals are most likely to be anemic; and 3) The enhanced growth performance in response to high levels of copper, but not zinc, added to antibiotic-free diets is dependent on an adequate iron status in the weaned pig. Contact: Mark J. Estienne, Ph.D. 757-807-6551 or mestienn@vt.edu.

Keywords: iron, copper, zinc, weaning, pig

Introduction:

Iron deficiency anemia can develop rapidly in suckling pigs because little iron is stored in the newborn animal; sow colostrum and milk have relatively insignificant iron concentrations; and modern genotypes have the capacity for extremely rapid growth rates. To prevent anemia, it is a standard industry practice to provide supplemental iron to newborn pigs, with intramuscular injections of 100 to 200 mg iron dextran common (Almond et al., 2017). Recent research, however, has demonstrated that many pigs, particularly the largest, fastest-growing animals in a litter, are iron deficient at weaning despite having received iron supplementation during the first week of life (Bhattarai and Nielsen, 2015a; Perri *et al.*, 2016). Anemic pigs at weaning have slower growth rates during the nursery phase of production (Bhattarai and Nielson, 2015b; Perri *et al.*, 2016). An additional iron treatment at weaning could be important, particularly for nursery pigs that consume diets supplemented with pharmacological levels of copper or zinc to enhance growth performance because these trace minerals have been shown to decrease liver iron concentrations and cause anemia (Cox and Hale, 1962). On commercial farms, elevated concentrations of zinc oxide (> 2000 ppm) in nursery diets are associated with a greater risk of anemia in pigs (Perri et al., 2016).

Objectives:

The objectives of the research were to determine the effects of iron injections at weaning on hematology characteristics and growth performance in nursery pigs fed antibiotic-free diets containing elevated levels of copper (Experiment 1) or zinc (Experiment 2).

Materials and Methods:

General. The protocols for these experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Virginia Tech (Blacksburg, VA).

Duroc-sired male and female pigs farrowed by Yorkshire x Landrace sows were employed. Within 24 hours after birth, pigs were ear notched for identification, weighed, and needle teeth were clipped and tails docked. All pigs also received an intramuscular injection of 100 mg iron hydrogenated dextran (Iron-100; Durvet, Inc., Blue Springs, MO) in the neck muscle behind the ear using a 20 gauge, 1.27 cm-long needle. At seven days of age, boar pigs were bilaterally castrated using a sterile scalpel.

At weaning pigs were moved to an environmentally-controlled nursery unit. Pen floors were galvanized steel bar slats. Nursery pens measured 4 x 3 feet and each contained a nipple drinker and a stainless steel feeder with four feeding spaces.

Study Designs. Pigs were weaned at approximately 21 days of age, weighed, and divided into equal groups of large and small pigs. Twelve pigs from each of the two size categories were placed three pigs/pen in six blocks of eight pens each, with litter of origin and sex balanced across pens. Pens were allocated to a 2 x 2 x 2 factorial arrangement of treatments. The factors were: 1) size of pig (large or small); 2) number of iron treatments (one intramuscular injection of 100 mg iron dextran within 24 hours after birth or one injection within 24 hours after birth and an additional injection of 100 mg at weaning); and, 3) level of dietary copper (5 or 250 ppm) (Experiment 1) or zinc (80 or 2,000 ppm) (Experiment 2). In each of the two experiments there were six replicate pens per treatment combination with 3 pigs/pen (total of 48 pens and 144 pigs for each of Experiment 1 and Experiment 2).

Experimental diets. Pigs were allowed ad libitum access to a three phase feeding regimen with all diets meeting the NRC (2012) requirements for the various nutrients and copper or zinc adjusted as indicated above. 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 (Experiment 1) or Zinc oxide (Experiment 2) (Maximo 720; Zinc Nacion, Monterrey, N.L, Mexico), or an equal amount of ground corn were added to the basal diet to create the trace mineral-supplemented or control diets, respectively.

Data and sample collection, and blood assay. Pigs were weighed at weaning and then weekly for 49 days. Average daily gain (**ADG**) was determined. Feed additions were recorded so that average daily feed intake (**ADFI**) and the ratio of gain to feed (**GF**), could be calculated.

Blood samples from a barrow weighing closest to the average initial weight of pigs in each pen, was collected at weaning (before the second injection of iron was administered to appropriate pigs), and at days 7 and 49 post-weaning. The same pig was used for each collection. On each occasion, animals were placed supine on a v-board and approximately 7 mL of blood collected via jugular venipuncture (20 gauge, 2.54 cm-long needle) using Vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) into a tube containing EDTA. Blood was used for complete blood counts conducted using a Coulter Multisizer 3 cell counter (Beckman Coulter, Inc., Brea, CA) by the Animal Laboratory Services of the Virginia-Maryland College of Veterinary Medicine (Blacksburg, VA). The following hematological determinations are reported: number of red blood cells, reticulocytes, and platelets; hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, and red blood cell distribution width.

Statistical Analysis. Data were subjected to ANOVA using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Average daily gain, ADFI and GF were analyzed using a model that included pig size, number of iron treatments, diet and all possible 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 possible 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.

Table 1: Composition of zinc oxide and control diets fed to nursery pigs for 49 days*

	Dietary Phase:	**I**	**II**	**III**
Feed Component, %	**Days fed:**	**0 - 7**	**7 – 21**	**21 - 49**
Ground corn		41.95	54.76	64.76
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
D,L-methionine		0.12	0.06	0.06
Vitamin-trace mineral†		0.50	0.50	0.50
Trace Mineral or ground corn		0.28	0.28	0.28
Totals		**100.00**	**100.00**	**100.00**
Calculated analysis, %				
Crude protein		20.55	20.31	19.55
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 (Experiment 1) or Zinc Oxide (Experiment 2), or control diets were prepared by mixing copper sulfate or zinc oxide (Maximo 720; Zinc Nacion, Monterrey, N.L, Mexico) or ground corn, respectively, with basal diet consisting of the major portion of the ground corn and all other common ingredients.

†ANS Swine Breeder Premix manufactured for Agri-Nutrition Services, Inc. (Shakopee, MN).

Results:

Incidence of anemia in pigs at weaning and subsequent growth. During Experiments 1 and 2**,** blood samples were collected from 48 large (17.5 ± 0.3 pounds body weight; LS means ± SE) and 48 small (12.5 ± 0.4 pounds body weight) barrows at weaning (prior to the second treatment with iron dextran in appropriate individuals). Six blood samples clotted before laboratory analyses were conducted, so data presented here represents a total of 90 pigs (n = 45 barrows/size group). More (P < 0.01) large (24/45, 53.3%) than small (7/45, 15.6%) pigs were classified as anemic by having hemoglobin concentrations of less than 9 g/dL (Friendship *et al.*, 1984). Across size groups, weaning weights were greater (P = 0.04) in anemic versus non-anemic pigs (15.5 ± 0.4 pounds versus 14.5 ± 0.4 pounds, respectively). Hemoglobin concentrations were greater (P < 0.01) in non-anemic (10.5 ± 0.1 g/dL) versus anemic (8.2 ± 0.2 g/dL) individuals, and tended to be greater (P = 0.07) in small pigs (9.6 ± 0.2 g/dL) versus large pigs (9.1 ± 0.1 g/dL). Hematocrit was greater (P < 0.01) in non-anemic (35.5 ± 0.4%) compared to anemic (28.5 ± 0.7%) individuals.

In barrows that were blood sampled, ADG during the first seven days post-weaning was affected by an interaction (P = 0.04) of anemic status and iron treatment at weaning (Figure 1). The ADG of anemic and non-anemic pigs that received iron treatment at weaning were similar (P > 0.05). In contrast, non-anemic pigs not receiving an iron treatment at weaning had greater (P < 0.05) ADG compared to their anemic counterparts. For the overall trial (weaning to day 49 post-weaning), large pigs at weaning had greater (P < 0.01) ADG compared to small pigs at weaning (1.21 ± 0.03 and 1.02 ± 0.04 pounds/day, respectively). There were, however, no effects of anemic status (P = 0.99), iron treatment at weaning (P = 0.86), or interactions of main effects (P > 0.16) on overall ADG.

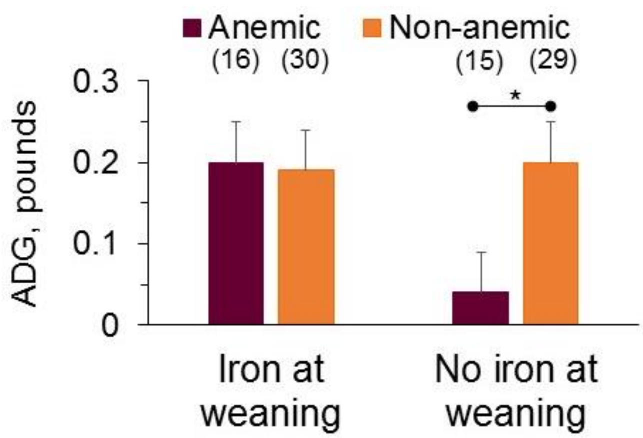

Figure 1. Average daily gain (ADG) ± SE from weaning to day 7 post-weaning in barrows (n = 90 pigs) classified as anemic (hemoglobin concentrations of less than 9.0 g/dL in blood collected at weaning) or non-anemic and that received iron dextran (100 mg intramuscularly) or no iron dextran at weaning. Numbers in parenthesis above each bar represent number of pigs in each group. There was an interaction of anemia status and iron treatment at weaning (P = 0.04) and ADG in non-anemic pigs that received no iron treatment at weaning was greater (, P < 0.05) compared to their anemic counterparts.*

Experiment 1: Effect of iron treatment at weaning on hematology characteristics and growth in pigs fed diets supplemented with 250 ppm copper.

Hematology characteristics were not affected by the main effect of iron treatment (at birth or at birth and at weaning) with two exceptions. Hematocrit was greater (P = 0.05) and platelet concentration was less (P = 0.05) in pigs that received iron at weaning (37.4 ± 0.9% and 379.6 ± 32.2 x 10^3/μL, respectively) versus pigs that did not (35.7 ± 0.9% and 448.9 ± 32.2 x 10^3/μL, respectively). The concentration of red blood cells was affected by the main effect of day ($P < 0.01$), and across treatments was 6.07 ± 0.09 x 10^6/μL at weaning, increased ($P < 0.05$) to 7.13 ± 0.08 x 10^6/μL on day 7 post-weaning, and then remained similar ($P > 0.05$) to day 49 post-weaning (7.26 ± 0.09 x 10^6/μL). The concentrations of platelets was also affected by day ($P < 0.01$), but across treatments was 530.2 ± 32.5 x 10^3/μL at weaning, decreased ($P < 0.05$) to 404.4 ± 31.8 x 10^3/μL at day 7 post-weaning, and decreased (P < 0.05) further to 308.3 ± 33.1 x 10^3/μL on day 49 post-weaning. Hemoglobin concentrations ($P = 0.02$), hematocrit ($P = 0.02$), and mean corpuscular volume ($P = 0.04$) were greater in pigs fed control diets (11.4 ± 0.3 g/dL, 37.5 ± 0.9%, 54.8 ± 1.1 fL, respectively) compared to pigs fed diets containing 250 ppm copper (10.8 ± 0.3 g/dL, 35.7 ± 0.9%, 52.6 ± 1.1 fL, respectively).

Hemoglobin concentrations, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, red blood cell distribution width, and reticulocyte concentration were affected ($P < 0.01$) by the interaction of pig size and day as shown in Figure 2.

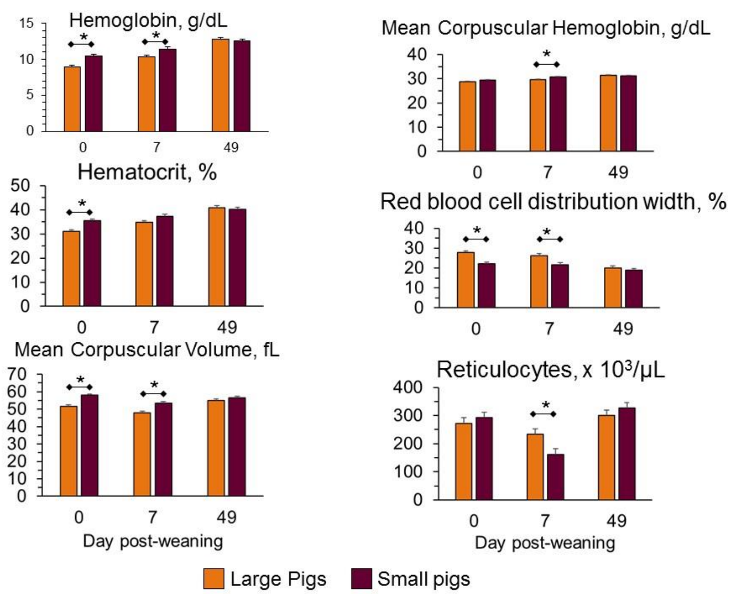

Figure 2. Hematological characteristics (LS means ± SE) at weaning (day 0), and days 7 and 49 post-weaning in barrows classified as large or small at weaning. There was an interaction of pig size and day (P < 0.01). Within days values marked with a horizontal bar and "" differ (P < 0.05) for large and small pigs.*

Mean corpuscular volume ($P = 0.01$) and reticulocyte concentrations ($P < 0.01$) were affected by an interaction between iron treatment (iron at birth versus iron at birth and at weaning) and day as shown in Figure 3.

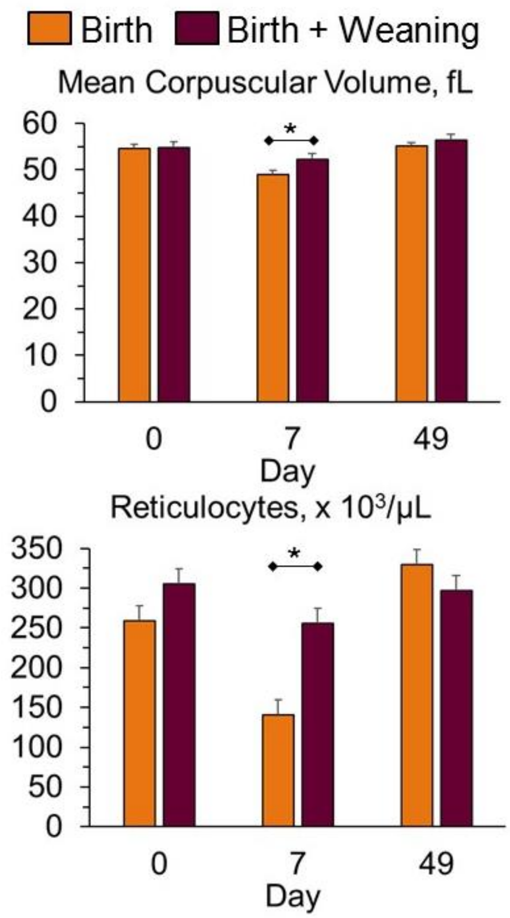

Figure 3. Hematological characteristics (LS means ± SE) at weaning (day 0), and days 7 and 49 post-weaning in barrows receiving iron treatment (100 mg intramuscular) at birth or at birth and at weaning. There was an interaction of iron treatment and day (P < 0.01). Within days values marked with a horizontal bar and "" differ (P < 0.05).*

From day 0 (weaning) until day 7 post-weaning, ADG and GF were affected (P = 0.04) by an interaction between treatment at weaning (in other words, iron or no iron) and diet (copper-supplemented or control). For pigs fed the copper diet, animals treated with iron at weaning had greater (P < 0.05) ADG and GF than animals not treated with iron. The additional iron treatment at weaning had no effect (P > 0.05) on ADG or GF in pigs consuming the control diet (Figure 4). Average daily feed intake was affected by diet and was greater for pigs fed copper compared to controls (0.56 ± 0.02 versus 0.48 ± 0.02 pounds per day, respectively).

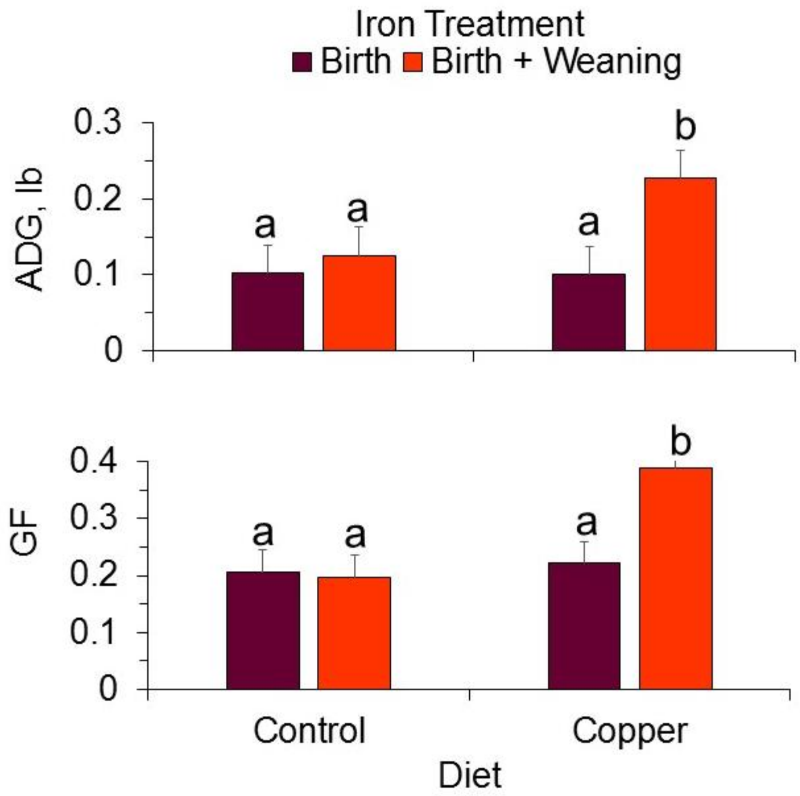

Figure 4. Average daily gain (ADG) ± SE and the gain to feed ratio (GF) from weaning to day 7 post-weaning in pigs that received iron dextran (100 mg intramuscularly) at birth or at birth and at weaning, and that received a control or copper supplemented (250 ppm) nursery diet. There was an interaction of iron treatment and diet on ADG and GF ($P = 0.04$). Bars accompanied by different superscripts differ ($P < 0.05$).

From day 0 until day 49 post-weaning (entire trial), ADG ($P = 0.02$) and ADFI ($P = 0.04$) were affected by an interaction between iron treatment and diet. For pigs fed the copper diet, animals treated with iron at weaning had greater ($P < 0.05$) ADG and ADFI than animals not treated with iron. The additional iron treatment at weaning had no effect ($P > 0.05$) on ADG or ADFI in pigs consuming the control diet (Figure 5).

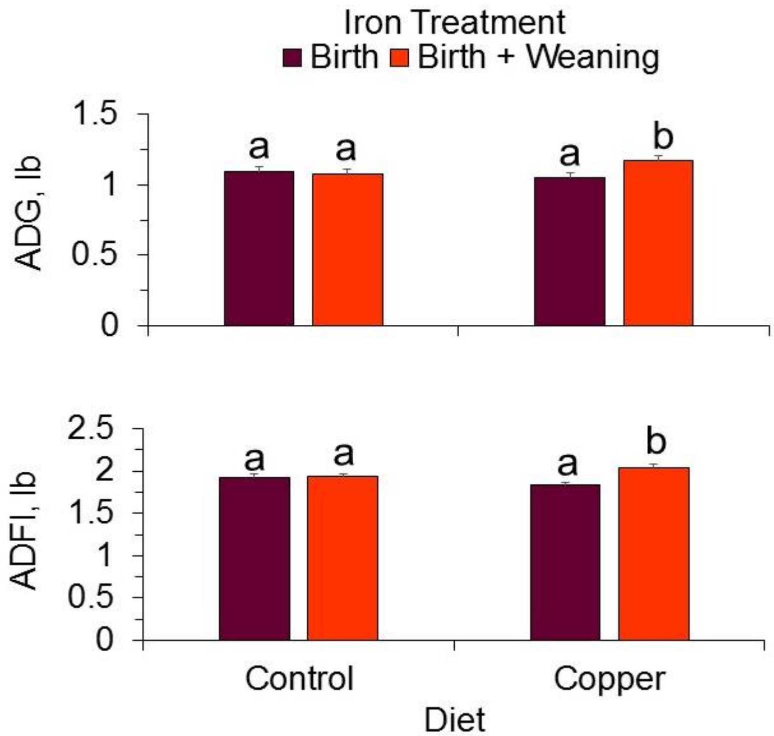

Figure 5. Average daily gain (ADG) ± SE and average daily feed intake (ADFI) from weaning to day 49 post-weaning in pigs that received iron dextran (100 mg intramuscularly) at birth or at birth and at weaning, and that received a control or copper supplemented (250 ppm) nursery diet. There was an interaction of iron treatment and diet on ADG (P = 0.02) and ADFI (P = 0.04). Bars accompanied by different superscripts differ (P < 0.05).

Experiment 2: Effect of iron treatment at weaning on hematology characteristics and growth in pigs fed diets supplemented with 2000 ppm zinc. Hematology characteristics were not affected ($P > 0.10$) by the number of iron treatments. Mean corpuscular hemoglobin tended to be greater ($P = 0.06$) in zinc-supplemented pigs compared to controls, but diet did not affect ($P > 0.10$) other hematology characteristics.

Hemoglobin concentrations ($P < 0.01$), hematocrit ($P = 0.05$), mean corpuscular volume ($P < 0.01$), mean corpuscular hemoglobin concentration ($P < 0.01$), and red blood cell distribution width ($P < 0.01$) were affected, and the concentration of red blood cells tended to be affected ($P = 0.06$) by the interaction of pig size and day (Figures 6 and 7). For large size pigs, red blood cell number tended to increase ($P < 0.06$) from day 0 to day 7, and then remained similar ($P > 0.06$) until day 49; Mean corpuscular hemoglobin concentration increased ($P < 0.05$) from day 0 to day 7, and further increased ($P < 0.05$) to day 49 (Figure 6). For small size pigs, the number of red blood cells tended to increase ($P < 0.06$) from day 0 to day 7, and from day 7 to day 49 ($P < 0.06$); Mean corpuscular hemoglobin concentrations increased from day 0 to day 7 ($P < 0.05$), and then remained similar ($P > 0.05$) until day 49 (Figure 6). Hemoglobin concentration and hematocrit were less ($P < 0.05$) in large- versus small-size pigs on day 0, but not on other days ($P > 0.05$); Mean corpuscular volume was less ($P < 0.05$), and red blood cell distribution width was greater ($P < 0.05$), in large size pigs versus small size pigs on days 0 and 7, but not on day 49 ($P > 0.05$) (Figure 7).

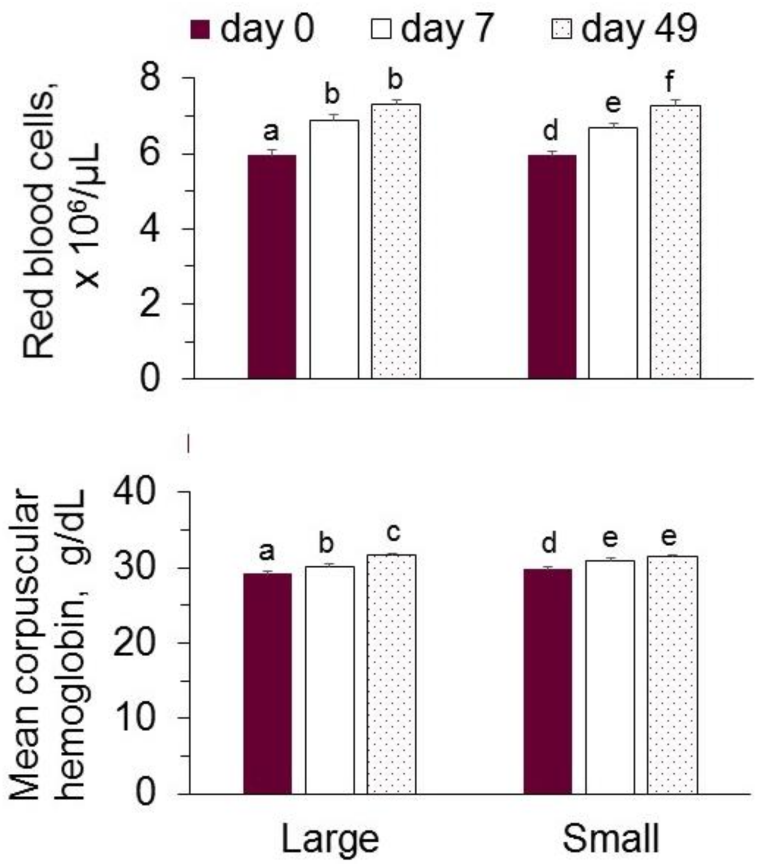

Figure 6. Red blood cell and mean corpuscular hemoglobin concentrations in large and small pigs at weaning (day 0) and days 7 and 49 post-weaning. Mean corpuscular hemoglobin concentration was affected (P < 0.01) and red blood cell concentration tended to be affected (P = 0.06) by an interaction of pig size and day. Within pig size for each hematology characteristic, bars with different superscripts differ (P < 0.05 for mean corpuscular hemoglobin concentration and P = 0.06 for red blood cell concentration).

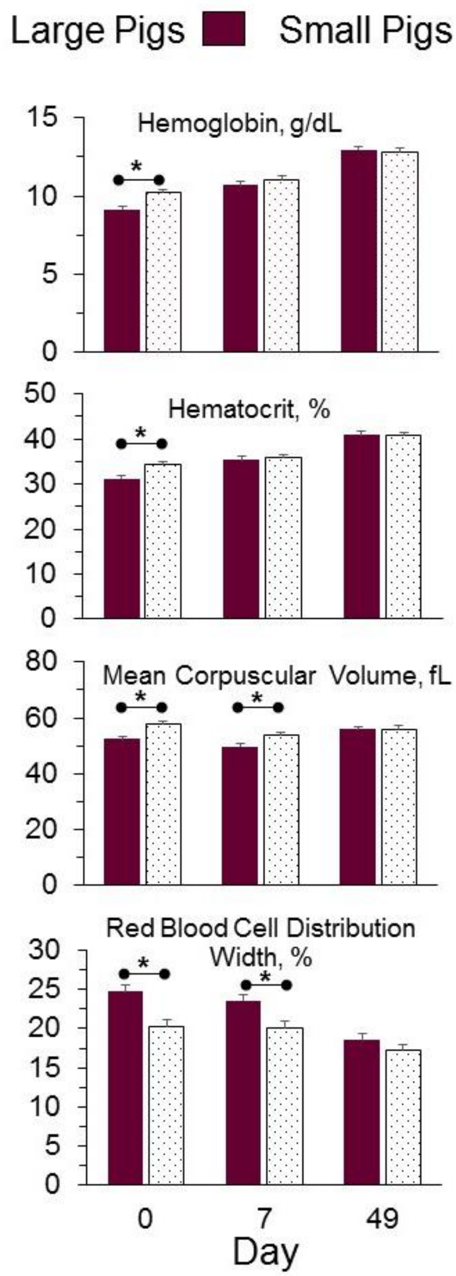

Figure 7. Hematology characteristics in large and small pigs in blood collected at weaning (day 0) and at days 7 and 49 post-weaning. Hemoglobin concentrations ($P < 0.01$), hematocrit ($P = 0.05$), mean corpuscular volume ($P < 0.01$), and red blood cell distribution width ($P < 0.01$) were affected by the interaction of pig size and day. For each characteristic, days on which values differed ($P < 0.05$) for large and small pigs are indicated by a horizontal bar and "".*

Table 3 summarizes growth performance in nursery pigs as affected by the main effects of size of pig, iron treatments, and diet. From day 0 (weaning) to 7, size of pig did not affect ADG ($P = .15$) or ADFI ($P = .12$), but GF was greater ($P < .01$) in the small-sized individuals. For the overall trial (day 0 to 49 post-weaning), ADG and ADFI were greater ($P < .01$) in large size pigs, and GF was greater ($P < .01$) in small size animals. In general, there were no effects of number of iron injections on growth performance measures. From day 0 to 7, zinc-fed pigs had greater ($P < .01$) ADG and GF, but similar ($P = .14$) ADFI compared to controls. Over the course of the trial, pigs fed diets supplemented with zinc had greater ADFI ($P < .01$) but similar ADG ($P = .44$) and GF ($P = .23$) compared with controls.

Table 3. Body weights and growth performance of large and small nursery pigs injected once or twice with iron dextran and fed control (Con) or zinc supplemented diets for 49 days

	Size of pig				Number of iron injections				Diet			
Items:	**Large**	**Small**	**SE**	***P***	**One**	**Two**	**SE**	***P***	**Con**	**Zinc**	**SE**	***P***
No. of pens[1]	24	24	---	---	24	24	---	---	24	24	---	---
Day 0 to 7												
Gain, lb/day	0.20	0.24	0.02	0.15	0.22	0.22	0.02	0.74	0.18	0.26	0.02	< **0.01**
Feed intake, lb/day	0.62	0.55	0.02	0.12	0.59	0.57	0.02	0.66	0.55	0.62	0.02	0.14
Gain:Feed	0.32	0.44	0.04	0.01	0.36	0.40	0.04	0.37	0.31	0.45	0.04	< **0.01**
Overall, Day 0 to 49												
Gain, lb/day	1.21	1.10	0.02	< **0.01**	1.17	1.14	0.02	0.36	1.14	1.17	0.02	0.44
Feed intake, lb/day	2.31	2.00	0.02	< **0.01**	2.13	2.18	0.04	0.37	2.07	2.24	0.03	< **0.01**
Gain:Feed	0.51	0.55	0.01	< **0.01**	0.54	0.53	0.01	0.40	0.54	0.53	0.01	0.23

[1]Each pen contained three pigs

Discussion:

Incidence of anemia in pigs at weaning and subsequent growth. Iron is a requisite component of hemoglobin, a protein molecule in red blood cells that carries oxygen from the lungs to bodily tissues and returns carbon dioxide from tissues back to the lungs. If iron levels in the body are inadequate to maintain a normal concentration of hemoglobin in the blood, iron deficiency anemia occurs. In the neonatal pig, iron deficiency anemia can be prevented by parenteral administration of iron (Ullrey *et al.*, 1959), and on modern swine farms, an intramuscular injection of iron dextran given within a few days after birth is common. The timing, dosage, and number of injections of iron dextran, however, varies widely among commercial operations (Almond *et al.*, 2017). Moreover, it is evident that despite treatment with iron early in life, a significant number of pigs, particularly the fastest growing animals within a litter, are iron deficient or anemic at weaning (Bhattarai *et al.*, 2015; Perri *et al.*, 2016).

For the current experiments, pigs with hemoglobin concentrations of less than 9.0 g/dL were classified anemic (Friendship *et al.*, 1984). Based on that criterion, a greater proportion of large pigs (53.3%) was classified as anemic at weaning compared with small pigs (15.6%). These results are consistent with previous studies by Bhattarai *et al.* (2015) and Perri *et al.* (2016) that also demonstrated an increased risk of anemia at weaning in larger, faster-growing pigs. The overall percentage of pigs in the current study that were classified anemic (34.4%) was greater than the proportion so classified (6%) in the previous study (Perri *et al.*, 2016), although in both investigations, the same criterion was used to identify the anemic condition (hemoglobin concentrations of less than 9.0 g/dL). Perhaps this difference reflects the greater dose of iron dextran (200 mg) administered at birth on the commercial farms in the study by Perri *et al.* (2016), compared to the dose (100 mg) used in the current experiments. Also, it is important to note that the proportion of pigs classified as anemic in the current study (34.4%) was similar to the proportion of pigs (34.8%) reported as anemic or iron deficient in the Perri *et al.* (2016) report.

The design of our study allowed us to look at the relationship between hematology characteristics and nursery growth in a small subset of anemic and non-anemic pigs that received either a single treatment of 100 mg iron (at birth) or treatment with 100 mg iron at both birth and weaning. Daily gain during the first seven days post-weaning was 40% greater in the non-anemic versus anemic pigs. Consistent with this finding, blood hemoglobin concentrations and other hematological characteristics such as the number of red blood cells and hematocrit in pigs at weaning were positively correlated with growth rate during the nursery phase of production (Bhattarai and Nielsen, 2015). Our data also show that the negative effect of iron deficient anemia on growth early post-weaning was mitigated by treatment with 100 mg iron dextran at weaning.

Dietary trace minerals such as copper and zinc have been shown to decrease liver iron concentrations and cause anemia (Cox and Hale, 1962). Consistent with the hypothesis that elevated levels of copper negatively impact iron absorption, in Experiment 1, hemoglobin concentrations and hematocrit, were greater in pigs fed control diets compared to pigs fed diets containing 250 ppm copper. In contrast, with the exception of a tendency for mean corpuscular hemoglobin concentrations to be greater in zinc-fed pigs than controls, diet did not affect hematology characteristics in Experiment 2. That hemoglobin concentrations were similar between groups suggests that the zinc-supplemented diets did not have an overt effect on iron absorption and utilization. Our results are in general agreement with a previous study (Abonyi et al., 2015) in which hematology profiles of weaned pigs fed pharmacological levels of dietary zinc were similar to control-fed animals with the exception of an increased percentage of lymphocytes.

In general, hemoglobin concentrations and other hematology characteristics were not dramatically affected by the additional iron injection at weaning in the experiments reported here. A second injection of 200 mg iron dextran 1 or 7 days before weaning at 21 or 28 days of age also failed to consistently increase hemoglobin concentrations in a case study conducted on five commercial pig farms (Almond et al., 2017). In pigs that received 200 mg of iron dextran within 24 hours after birth, hemoglobin concentrations and hematocrit increased only slightly after an additional treatment with iron at weaning (17 days of age), and by 21 days post-weaning were actually less compared with pigs that received iron only at birth (Peters and Mahan, 2008). A second injection of iron dextran at 21 days of age increased hemoglobin concentration, but not hematocrit, in pigs at weaning (28 days of age) but at 21 days post-weaning, both hematology characteristics were not affected by the second treatment (Kamphues et al., 1992). For pigs weaned and blood sampled at 34 days of age, a second injection of 200 mg iron dextran at day 20 of age also increased hemoglobin concentrations (Haugegaard et al., 2008).

Positive growth responses in nursery pigs provided pharmacological concentrations of copper or zinc oxide have been well-documented, and in most studies, experimental diets have been fed for 28 days (Smith et al., 1997; Hill et al., 2001a,b; Case and Carlson, 2002). For example, Hill et al. (2001b) conducted a study during which weaned pigs were fed diets containing zinc oxide at levels of 0, 500, 1,000, 2,000, or 3,000 ppm for 28 days. As dietary zinc oxide increased, pigs weaned at less than 15 days of age had greater ADG, ADFI and GF, while pigs weaned at greater than 20 days of age displayed greater ADG and ADFI. Responses for both pig groups plateaued at 2,000 ppm (Hill et al., 2001).

For pigs weaned at 21 days of age in the current experiments, ADG and ADFI from day 0 until day 49 post-weaning were affected by an interaction between iron treatment and diet. For pigs fed the copper diet, animals treated with iron at weaning had greater ADG and ADFI than animals not treated with iron. In contrast, the additional iron treatment at weaning had no effect on ADG or ADFI in pigs consuming the control diet. Finally, increased ADG and GF in response to 2,000 ppm zinc was limited to the first 7 days of the experiment.

In summary,

1. Consistent with previous work at Virginia Tech (Callahan *et al.*, 2017) and other institutions, a significant proportion of pigs were anemic at weaning, despite having received intramuscular iron shortly after birth;

2. Within litters, the larger, faster growing individuals were most likely to be anemic;

3. Anemic pigs displayed reduced growth performance in the nursery, particularly during the first week post-weaning; and,

4. Enhanced growth performance in response to high levels of copper, but not zinc, added to antibiotic-free diets was dependent on an adequate iron status in the weaned pig.

(References for cited literature available on request)