Article

The relationship between hemoglobin levels at weaning and growth performance and antibody response in nursery pigs

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Abstract — The objectives of this study were to determine whether weaned pigs with low hemoglobin (Hb) concentrations grow more slowly and produce poorer antibody response to vaccination compared to pigs with normal Hb concentrations, and to study the association between high levels of zinc oxide in feed and continued anemia in pigs during the nursery phase. At weaning, pigs were classified as anemic (< 90 g/L of Hb), iron deficient (90 to 110 g/L Hb), or normal (> 110 g/L Hb). Pigs were vaccinated twice against *Mycoplasma hyopneumoniae* and antibody response was measured 3 weeks after the last vaccination. Two trials were performed with diets containing 150 mg/kg of zinc oxide and a third trial was conducted with pigs fed a diet containing 3000 mg/kg of zinc oxide for 2 weeks post-weaning. Hemoglobin status didn't impact growth rate or antibody response to vaccination. High zinc levels in the feed did not alter the pattern of Hb concentrations in pigs post-weaning.

Résumé — Relation entre les niveaux d'hémoglobine au sevrage et les performances de croissance et la réponse en anticorps chez des porcs en pouponnière. Les objectifs de la présente étude étaient de déterminer si les porcs sevrés avec de faibles concentrations en hémoglobine (Hb) avaient une croissance plus lente et produisaient moins d'anticorps en réponse à une vaccination comparativement à des porcs avec une concentration normale de Hb, et d'étudier l'association entre des niveaux élevés d'oxyde de zinc dans la moulée et une anémie continue chez les porcs durant la période en pouponnière. Au sevrage les porcs furent classés comme anémiques (< 90 g/L de Hb), déficients en fer (90 à 110 g/L de Hb) ou normaux (> 110 g/L de Hb). Les porcs furent vaccinés deux fois contre *Mycoplasma hyopneumoniae* et la réponse en anticorps mesurée 3 semaines après la dernière vaccination. Deux essais ont été effectués avec des diètes contenant 150 mg/kg d'oxyde de zinc et un troisième essai mené avec des porcs nourris avec une diète contenant 3000 mg/kg d'oxyde de zinc pendant 2 semaines après le sevrage. Le statu en hémoglobine n'avait pas d'impact sur le taux de croissance ou la réponse en anticorps à la suite de la vaccination. Des niveaux élevés de zinc dans la moulée n'ont pas altéré le patron des concentrations de Hb chez les porcs en post-sevrage.

(Traduit par D[r] Serge Messier)

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Introduction

The first few weeks after weaning are stressful for pigs and this transition period is often characterized by poor feed intake, slow growth, and a high incidence of disease. This is a time when the pig's immune system is still maturing but passive immunity is disappearing, leaving the pig susceptible to disease challenges. In addition, on many farms, immunization procedures take place at weaning and their effectiveness relies on the pig being healthy and strong. It is therefore important that pigs entering the nursery are in a good nutritional state with a robust immune system. Recently, studies have reported that a proportion of large, fast-growing pigs are iron deficient or anemic at the time of weaning, despite the almost universal practice of injecting pigs intramuscularly at around 3 d of age with 100 or 200 mg of iron (1–3). Iron has been shown to be a nutrient related to health and immune function (4,5). Some researchers have reported that a second injection of 200 mg of iron close to weaning improves the pig's iron status in the first few weeks of the nursery stage and may result in faster growth (6), but other studies have shown no effect of iron status at weaning on growth and immune function (7).

Anemia has been shown to persist in the early nursery phase on certain farms, even though pigs are provided with starter diets enriched with iron (1,3). Researchers have suggested high levels of zinc oxide, commonly added to starter diets as a means

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Table 1. Formulated values of the 4 starter diets that were fed to pigs during the nursery phase of the study.

	Diet phase			
Component	Starter 1[a] (7.2 to 11.9 kg)[b]	Starter 2[a] (11.9 to 16.2 kg)	Starter 3 (16.2 to 20.1 kg)	Starter 4 (20.1 to 28.1 kg)
Crude protein (min) %	20	19.5	19	18
Crude fat (min) %	3.0	3.0	2.5	2.0
Crude fiber (max) %	2.5	3.5	3.0	3.5
Calcium (actual) %	0.83	0.8	0.69	0.66
Phosphorus (actual) %	0.67	0.66	0.57	0.55
Sodium (actual) %	0.3	0.25	0.2	0.2
Copper (actual) mg/kg	125	125	125	125
Zinc (actual) mg/kg	150	150	150	150
Vitamin A (min) IU/kg	10 000	10 000	10 000	10 000
Vitamin D (min) IU/kg	1500	1500	1500	1500
Vitamin E (min) IU/kg	70	70	65	65
Selenium (mg/kg)	0.5	0.5	0.5	0.5
Iron (mg/kg)	142	142	175	175
Lincomycin (mg/kg)	44	44	44	44
kilogram feed/pig[c]	6	6	8	14

[a] In trial 3 zinc oxide was increased to 3000 mg/kg in starter 1 and starter 2.
[b] Weights refer to the average weight of pigs while consuming the diet.
[c] Feed, kg/pig is based on the average recommendation of feed intake per animal for each diet.

Table 2. Hemoglobin (Hb) levels of pigs at 21 and 35 or 42 d post-weaning categorized on the basis of their iron status at weaning: anemic (Hb $<$ 90 g/L), iron deficient (Hb 90 to 110 g/L), normal (Hb $>$ 110 g/L).

	Anemic	Iron deficient	Normal
Trial 1[a]			
Hb at weaning (g/L)	77.0 ± 1.3[a]	100.2 ± 1.3[b]	122.1 ± 1.0[c]
Hb at 21 d post-weaning (g/L)	105.8 ± 2.7[a]	109.6 ± 1.9[a]	118.3 ± 2.3[b]
Hb at 42 d post-weaning (g/L)	130.5 ± 1.2	127.9 ± 1.4	128.0 ± 1.0
Trial 2[a]			
Hb at weaning (g/L)	81.8 ± 1.9[a]	98.8 ± 1.1[b]	127.0 ± 1.5[c]
Hb at 21 d post-weaning (g/L)	96.5 ± 2.7[a]	103.8 ± 1.8[a,b]	107.6 ± 1.2[b]
Hb at 42 d post-weaning (g/L)	120.9 ± 2.4	120.9 ± 1.9	121.4 ± 1.0
Trial 3[a]			
Hb at weaning (g/L)	75.0 ± 1.1[a]	101.2 ± 0.8[b]	123.5 ± 1.0[c]
Hb at 21 d post-weaning (g/L)	101.8 ± 1.0[a]	108.9 ± 1.0[b]	110.7 ± 0.9[b]
Hb at 35 d post-weaning (g/L)	125.1 ± 0.8	128.0 ± 1.0	127.8 ± 0.8

[a] The trials were similar except that pigs in trial 3 were fed the first 2 nursery diets containing 3000 mg/kg of zinc oxide. Values are expressed as mean ± standard error of the mean. Different superscripts denote statistical difference ($P < 0.05$).

of controlling bacterial enteric disease, might interfere with iron uptake and therefore contribute to the persistence of anemia in the post-weaning phase (1).

The objectives of this study were to: examine the relationship between iron status [as determined by hemoglobin (Hb) levels] at weaning and humoral immune response to vaccination of pigs against *Mycoplasma hyopneumoniae,* determine whether iron status at weaning has an effect on the growth rate of pigs in the nursery stage of production, and determine if the inclusion of high levels of zinc oxide (3000 mg/kg) in starter diets affects iron status post-weaning.

Materials and methods

The study was approved by the Animal Care Committee at the University of Guelph and conducted at the Arkell Swine Research Station. The Arkell herd is a high-health, closed-herd and is free of *M. hyopneumoniae.* Two trials were performed with weaned pigs fed starter diets (Table 1) with zinc included at 150 mg/kg in all diets. The study was repeated using a third trial in which weaned pigs were fed similar diets to the first 2 trials, but in starter 1 and starter 2 diets, zinc oxide was added at a rate of 3000 mg/kg. In the first 2 trials, pigs were selected from 22 litters (11 litters in each trial). About 2/3 of the pigs from the 22 litters (trial 1: $n = 70$, trial 2: $n = 75$) were placed into the study. The pigs in these litters that were not included in this study had been selected for a different research trial. The decision to remove pigs from trials 1 and 2 was based on a polymerase chain reaction (PCR) test for the porcine *Mucin 4* gene. The other research trial required pigs to be susceptible to enterotoxigenic *Escherichia coli* and therefore removed pigs that were positive for the *Mucin 4* gene. In the third trial all pigs from 22 litters were included ($n = 214$). In the first 2 trials pigs were followed for 6 wk post-weaning but in the third trial pigs were monitored for only 5 wk post-weaning.

In order to ensure pigs at weaning would have varying levels of Hb, 3 different iron supplementation regimens were employed. On the day after farrowing, all piglets were identified by an individual ear tag and assigned to 1 of the 3 iron dose

Table 3. Average daily gain (ADG) and antibody titers [sample to positive (S/P) ratio] to *Mycoplasma hyopneumoniae* vaccination in pigs categorized at weaning based on hemoglobin (Hb) levels: anemia (Hb < 90 g/L), iron deficient (Hb from 90 to 110 g/L), and normal (Hb > 110 g/L).

Trial 1[a]	Anemic (n = 23)	Iron deficient (n = 16)	Normal (n = 31)
Weight at weaning (kg)	7.8 ± 0.2[a]	7.6 ± 0.2[a]	6.3 ± 0.2[b]
Weight 42 d post-weaning (kg)	27.5 ± 0.6	28.0 ± 0.8	25.4 ± 0.7
ADG for 42 d in nursery (kg/d)	0.56 ± 0.01	0.58 ± 0.01	0.55 ± 0.01
S/P ratio for *M. hyopneumoniae* at 42 d post-weaning	0.45 ± 0.04	0.37 ± 0.04	0.43 ± 0.05
Trial 2[a]	**Anemic (n = 10)**	**Iron deficient (n = 20)**	**Normal (n = 45)**
Weight at weaning (kg)	7.2 ± 0.3[a]	6.2 ± 0.3[b]	6.2 ± 0.2[b]
Weight 42 d post-weaning (kg)	26.0 ± 1.1	25.6 ± 1.0	25.1 ± 0.5
ADG for 42 d in nursery (kg/d)	0.45 ± 0.02	0.46 ± 0.02	0.45 ± 0.01
S/P ratio for *M. hyopneumoniae* at 42 d post-weaning	1.18 ± 0.17	0.99 ± 0.11	0.88 ± 0.06
Trial 3[a]	**Anemic (n = 80)**	**Iron deficient (n = 63)**	**Normal (n = 71)**
Weight at weaning (kg)	6.2 ± 0.1[a]	6.4 ± 0.2[a]	5.6 ± 0.2[b]
Weight at 35 d post-weaning (kg)	22.1 ± 0.4	22.7 ± 0.5	21.3 ± 0.6
ADG for 35 d in nursery (kg/d)	0.46 ± 0.01	0.46 ± 0.11	0.45 ± 0.01
S/P ratio for *M. hyopneumoniae* at 35 d post-weaning	0.36 ± 0.03	0.37 ± 0.04	0.38 ± 0.04

[a]Values are expressed as mean ± standard error of the mean.
Different superscripts denote statistical difference ($P < 0.05$).

treatments through systematic random sampling. The treatment groups were: Low Dose — an IM injection at 3 d of age of 100 mg iron dextran (Uniferon 200; Pharmacosmos, Watchung, New Jersey, USA); Medium Dose — an injection of 200 mg iron dextran at 3 d of age; and High Dose — an injection of 200 mg of iron dextran at 3 d of age and repeated at 14 d of age. Creep feed was not provided in the farrowing room.

At weaning (~21 d of age) all piglets were vaccinated against *M. hyopneumoniae* (2 mL IM, Suvaxyn MP/HPS; Zoetis, Kirkland, Quebec) and revaccinated 3 wk later. In each trial, pigs were housed in a single nursery room and segregated by sex, placing gilts and barrows in separate pens. On average, 10 pigs were housed in each pen and Low, Medium, and High Dose pigs were evenly distributed among pens as much as possible. These treatment groups were only used as a means to ensure that some pigs would be anemic and some would have normal Hb levels. The focus of the study was not on the merits of the different pre-weaning treatments. Weaned pigs were provided access to feed *ad libitum*. Throughout the nursery phase, a total of 4 starter diets were fed (Table 1). Each starter diet was fed according to pig weight guidelines. Individual pig weights were taken at weaning, and at 3 wk and 6 wk post-weaning in trials 1 and 2. In trial 3, pigs were individually weighed at weaning and 5 wk later.

In trials 1 and 2, at weaning and 3 wk and 6 wk post-weaning, 2 blood samples were collected from each pig *via* the orbital sinus in order to measure the blood Hb and *M. hyopneumoniae* serum antibodies. In trial 3, blood samples were collected at weaning and at 3 and 5 wk post-weaning, but blood at 3 wk was only used for Hb determination. The whole blood sample was collected into a tube (BD Vacutainer; BD, Franklin Lakes, New Jersey, USA) containing an anticoagulant, ethylenediaminetetraacetic acid (EDTA), and submitted to the Animal Health Laboratory (AHL), University of Guelph within 3 h after collection to be analyzed for Hb using an ADVIA 2120/2120i Hematology system (Siemens Healthcare Diagnostics, Deerfield, Illinois, USA). The blood samples for serology were collected into a tube without anticoagulant. These blood samples were centrifuged at 2500 RPM for 20 min and sera were removed and placed into 2-mL tubes and stored at −20°C to be analyzed later for antibody response to *M. hyopneumoniae* vaccine.

Serum samples were submitted to AHL to test for antibodies to *M. hyopneumoniae* using an enzyme-linked immunosorbent assay (ELISA) (IDEXX M. Hyo Ab Test; IDEXX Laboratories, Westbrook, Maine, USA). The values of sample-to-positive (S/P) ratio provided by the manufacturer were used as an indicator of antibody response to the vaccine in pigs in the various treatment groups. The interpretation of the results according to the manufacturer of the ELISA is: seronegative for S/P ratio < 0.3, seropositive for S/P ratio > 0.4, and suspect for S/P ratio between 0.3 and 0.4.

Blood samples taken at weaning were used to categorize the pigs into 3 groups based on Hb levels as follows: "anemic" (Hb < 90 g/L), "iron deficient" (Hb 90 to 110 g/L), and "normal" (Hb > 110 g/L).

Statistical analysis

Data were entered into Microsoft Excel 2016 (Microsoft, Redmond, Washington, USA) Version 15.28, and were imported into STATA 14 (STATA for Mac; StataCorp, College Station Texas, USA). The mean, median, proportion, and standard error of the mean of each outcome including S/P ratio and seropositivity to *M. hyopneumoniae* vaccine, Hb levels (iron status), and growth rates (average daily gain) were described for each collection period.

A mixed-effects, multi-level linear regression method with sow (clustering) as a random effect was used to determine the relationship between antibody titers (S/P ratio) to *M. hyopneumoniae* vaccine and total average daily gain. A mixed-effects, multi-level linear regression method with sow (clustering) and test (repeated measure) as random effects were also used to determine the association of Hb level at weaning with weight within the 5- to 6-week time period pigs were in the nursery.

Results

The percentages of pigs in each treatment category that were anemic were 79.2, 10.6, and 0.08 for pigs treated with Low, Medium, and High Dose, respectively.

The mean Hb levels of pigs in each iron status category at weaning, and after 3 and 5 to 6 wk post-weaning are presented in Table 2. Anemic pigs and iron-deficient pigs improved their iron status during the nursery phase and the mean Hb levels for all 3 study groups in the 3 trials were in the normal range at 5 to 6 wk post-weaning. In trial 3, the first 2 starter feeds contained zinc oxide at a concentration of 3000 mg/kg, compared to 150 mg/kg in the starter diets used in trials 1 and 2. However, the pattern in all 3 trials was characterized by a decrease in Hb levels after 3 wk in the nursery for pigs that were categorized as normal at weaning but then a return to normal levels 2 to 3 wk later. Pigs that were categorized as anemic at weaning, however, demonstrated an increase in Hb levels in the first 3 wk after weaning but were, on average, iron deficient at 3 wk post-weaning and then normal at 5 to 6 wk post-weaning.

The growth performance in the nursery and the immune response to the *M. hyopneumoniae* vaccinations for pigs in each iron status category (anemic, iron deficient, and normal) at weaning for each of the 3 trials are shown in Table 3. Pigs at weaning that were classified as anemic were heavier than pigs with Hb levels in the normal range and this was a consistent finding in all 3 trials ($P < 0.05$). At the final weighing in all 3 trials, the mean weights of pigs in the 3 study groups were similar. There was no significant difference in the average daily gain (ADG) during the nursery phase among the iron status groups ($P > 0.05$). The immune response to vaccination as measured by an ELISA test using a S/P ratio was similar for the 3 study groups (anemic, iron deficient, and normal at weaning) in all 3 trials. All pigs were seronegative (S/P ratio < 0.3) for *M. hyopneumoniae* at weaning and at 3 wk post-weaning.

Discussion

Pigs that were anemic at weaning (Hb < 90 g/L) appeared to grow and respond to vaccination in a similar manner to pigs with adequate iron as defined by normal Hb levels (> 110 g/L). Although some previous studies (1,6–8) have reported an association between anemia and slower growth rate, our findings are in agreement with at least one other study that found no relationship between anemia at weaning and reduced growth in the nursery (5). However, the literature is consistent with respect to the relationship between the weight at weaning and iron status. In the present study, anemic pigs at weaning weighed more than pigs with normal Hb levels and this was true in all 3 trials. The explanation for this is that large, fast-growing pigs have a greater requirement for iron than small, slow-growing pigs (1,2). It is possible that the large pigs at weaning had the potential to grow much faster than other pigs in the nursery but were negatively affected by anemia. If the anemic large pigs in this study had been provided with adequate iron during the suckling period, they may have grown faster than the other pigs in the nursery phase. Further studies are required to investigate whether iron deficiency at weaning is limiting potential growth of the biggest pigs.

Similar to other studies (1,3,8), it was noted that many pigs were iron deficient after 3 wk of consuming starter diets containing > 140 mg of iron per kg of feed. Pigs with normal Hb levels at weaning tended to have lower levels of Hb 3 wk post-weaning. This may reflect the low consumption rate of starter feed for the first wk after weaning that many pigs experience (9) or it could reflect poor iron absorption from the intestine. Perri et al (1) found an association between farms that fed zinc oxide at high therapeutic levels (> 2000 mg/kg) and the prevalence of anemia in pigs 3 wk after weaning. These authors speculated that zinc interfered with iron absorption as described by Cox and Hale (10). In the present study, high levels of zinc in the diet didn't appear to alter Hb patterns, confirming the findings recently reported by Estienne et al (11).

The present study also found that there was no association between the iron status of pigs at weaning and their antibody response to *M. hyopneumoniae* vaccination. In human medicine Chandra and Saraya (12) examined humoral immunity in iron deficient children by measuring concentrations of serum immunoglobulins and antibody responses to tetanus toxoid and *Salmonella* Typhi vaccine and also noted that anemic individuals produced a normal response. This is in contrast to a study that found iron-deficient rats produced lower antibody titers against influenza virus than rats that were supplemented with recommended levels of dietary iron (13). Overall, studies that have investigated the relationship between iron status and immune function in newly weaned pigs have produced inconsistent results and this topic requires further investigation. One explanation for the lack of a negative impact with respect to iron deficiency in this study is that most of the anemic pigs were not severely affected and that as soon as they entered the nursery and had access to feed containing iron, they improved. Pigs that were anemic at weaning finished the nursery phase with similar Hb levels and similar weights to pigs that had normal Hb levels at weaning, suggesting that the iron deficiency at weaning was temporary and reversible, and did not appear to be associated with a negative impact.

Acknowledgments

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Book Review
Compte rendu de livre

Coping with Stress and Burnout as a Veterinarian

Hamilton N. Australian Academic Press Group Pty. Ltd. Samford Valley, Queensland, Australia. 2019. 173 pp. ISBN: 9781-9256-4419-7.

This amazing book entered my life at just the right time. On my way to lecture at the Atlantic Veterinary College (AVC) in late 2019 on just this subject it gave me many new insights into the mental health concerns in veterinary medicine. I was also impressed that 2 AVC individuals that attended the American Veterinary Medical Association (AVMA) workshop in this area had received copies of Dr. Hamilton's book at that time. I know they will utilize it in being leading lights for the students they serve during their undergraduate years and it will make a difference (maybe a life-saving one) in their future paths as veterinarians whatever their roles. Dr. Nadine Hamilton (the author) is a psychologist who helps professionals get on top of stress and psychological fatigue to avoid burnout and suicide. Her story details her early life trials in finding what she considers an appropriate place in society. The fact that one of her early decisions was to try veterinary medicine is an appropriate one because it instilled in her the desire to become an important mentor to those in our profession that have great need. The first part of this book is dedicated to the history on a global basis of mental health issues that have been long hidden within our profession. Learning in some ways how and why we struggle with such concerns, in what the public believes to be a perfect job, is important for us all to understand. Once you arrive at page 89, it is titled "Intervention." The remainder is a working model of how this book lives up to the claim on the cover of "An Evidence-Based Solution to Increase Wellbeing." For all who work in veterinary medicine and would like to begin to be part of the solution to mental health concerns in our profession, I would hope this book would be part of their library. For any in the veterinary profession (all members of any veterinary team), keeping a copy at hand for quick reference and potential solutions would be a good idea. While signs of mental health issues or even suicide should warrant immediate attention and a referral to a professional trained in these specific subjects, this fine book could be part of the early warning signs to seek such help.

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