An investigation of various hematological and biochemical parameters to assess the health of nursery pigs

by

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AN INVESTIGATION OF VARIOUS HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS TO ASSESS THE HEALTH OF NURSERY PIGS

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This thesis investigates hematological and biochemical parameters used to assess the health of nursery pigs. Beta-hydroxybutyrate (BHB) levels were measured using standard laboratory serum testing and a handheld meter to diagnose ketosis in newly weaned pigs. Pigs displaying chomping behaviour and thin pigs had higher BHB values than clinically normal pen-mates. The two diagnostic methods used were 94% correlated indicating good agreement.

Hemoglobin levels from 1095 pigs at weaning and 3-weeks post-weaning were measured. Anemic and iron deficient pigs were found at weaning on almost all farms and anemia was negatively associated with post-weaning growth. Additionally, a high level of zinc oxide (≥2000 mg/kg) in starter rations was associated with an increased likelihood of anemia post-weaning. These findings suggest that iron supplementation practices aren’t sufficient, particularly for large piglets. Future work is needed to gain a better understanding of zinc oxide usage and its association with post-weaning anemia.
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When someone you love becomes a memory, the memory becomes a treasure.

The greatest treasures are those invisible to the eye but found by the heart.

This thesis is dedicated in memory of my Zia Pierina, a life so beautifully lived
and a heart so deeply loved.

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CONTRIBUTIONS

Amanda Perri coordinated and participated in all the field work, collection of samples, data management and analyses, interpretation of the results, dissemination of the results, and was principal author of all the chapters and manuscript(s) arising from the chapters.

Dr. Terri O’Sullivan led the research projects, assisted and helped coordinate the field work, assisted with the field work and sample collections, helped interpreted results and provided critical feedback on all chapters.

Dr. Bob Friendship helped with coordinating the field work, interpreted results and provided critical feedback on all chapters.

Dr. John Harding assisted and helped coordinate the field work, helped interpreted results and provided critical feedback on all chapters.

Dr. Darren Wood helped coordinate biochemical research project, assisted with statistics and interpreting hematology and biochemistry values.

Mackenzie Slifierz helped with fieldwork, sampling and designing of the questionnaire.

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Emily Zakrjsek and Ryan Tenbergen helped with the field work, collecting samples and validating data.

Karen Richardson helped with scheduling farms and vehicles.

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# TABLE OF CONTENTS

LIST OF TABLES ............................................................................................................. VI
LIST OF FIGURES .......................................................................................................... VII
LIST OF ABBREVIATIONS ............................................................................................ VIII

## CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction ........................................................................................................ 1
  1.1.1 Production Practices ....................................................................................... 3
  1.1.2 Sanitation ......................................................................................................... 5
  1.1.3 All-In/All-Out Production Protocols ................................................................. 6
  1.1.4 Ambient Temperature ..................................................................................... 7
  1.1.5 Ventilation and Relative Humidity ................................................................. 9
  1.2 Weaning Stress ................................................................................................... 9
  1.2.1 Physiological Stress .......................................................................................10
  1.2.2 Social Stress ..................................................................................................13
  1.2.3 Nutritional Stress ..........................................................................................13
  1.2.4 Environmental Stress ..................................................................................15

1.3 Post-weaning Anorexia ....................................................................................... 16

1.4 Periweaning Failure to Thrive Syndrome (PFTS) ............................................. 17

1.5 Ketones and Ketosis .......................................................................................... 20

1.6 Iron ...................................................................................................................... 21
  1.6.1 Iron Deficiency and Anemia .......................................................................... 22
  1.6.2 Etiology of Iron Deficiency and Anemia in Pigs ........................................ 22
  1.6.3 Immunocompetence ...................................................................................... 24
  1.6.4 Clinical Signs of Iron Deficiency and Anemia ............................................ 24
  1.6.5 Indicators of Iron Deficiency and Anemia .................................................... 25
    1.6.5.1 Hemoglobin ......................................................................................... 25
    1.6.5.2 Packed Cell Volume ............................................................................. 26
    1.6.5.3 Erythrocytes ....................................................................................... 27
    1.6.5.4 MCV, MCH, MCHC ........................................................................... 27
    1.6.5.5 Serum Iron & Serum Total Iron-Binding Capacity ................................ 28
  1.6.6 Oral Iron Supplementation Practices and Dosing ........................................ 28
  1.6.7 Parenteral Iron Supplementation Practices and Dosing .............................. 32
  1.6.8 Reassessment of Iron Supplementation Protocols ....................................... 35
  1.6.9 Iron Toxicity .................................................................................................. 36

1.7 Zinc ...................................................................................................................... 37

1.8 Hematological and Biochemical Analysis ......................................................... 37

1.9 Thesis Objectives ................................................................................................ 38

References .................................................................................................................. 41
LIST OF TABLES

Table 2.1: Number and percentage of ketosis by pig group (Control, Chomp, Thin) using different beta-hydroxybutyrate (BHB) cut-off values .......................................................... 72

Table 2.2: Mean ranks of beta-hydroxybutyrate (BHB) values by pig group for both the Rx Monza and Precision Xtra diagnostic tools................................................................. 73

Table 2.3: Pairwise contrasts of mean ranks of beta-hydroxybutyrate (BHB) Rx Monza values ....................................................................................................................... 74

Table 2.4: Mean ranks of beta-hydroxybutyrate (BHB) values by province obtained using the Rx Monza and Precision Xtra diagnostic tools.................................................. 75

Table 2.5: Mean ranks of beta-hydroxybutyrate (BHB) values by farms and province for both the Rx Monza and Precision Xtra diagnostic tools............................................. 76

Table 3.1: Summary of farm production parameters, iron supplementation protocols, and iron status of piglets at the time of weaning and at 3 weeks post-weaning from 20 commercial swine farms in Ontario ............................................................................................................................ 115

Table 3.2: The final model* illustrating the effect of iron deficiency and/or anemia at weaning on 3-week post-weaning body weight (kg) .................................................................................. 118

Table 3.3: Eight individual models* illustrating the associations between various iron status indicators and the body weight category at 1 to 2 days prior to weaning from 1095 piglets from 20 Ontario commercial swine farms................................................................. 119

Table 3.4: The mean (±SD) of various iron analytes from 1095 piglets sampled prior to weaning* from 20 Ontario commercial swine farms ........................................................................ 120

Table 3.5: Model* assessing the association between zinc concentration in feed and odds of anemia in piglets at 3-weeks post-weaning on twenty commercial farms ................. 121

Table 4.1: Complete blood count (CBC) and iron profile references valuesa of Ontario pigs sampled 1 to 2 days prior to weaningb on commercial farms .......................................................... 134

Table 4.2: Biochemistry reference valuesa of Ontario pigs 1 to 2 days prior to weaningb on twenty commercial farms ........................................................................................................ 135
LIST OF FIGURES

Figure 2.1: Number of normal (non-ketotic) and ketotic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on Rx Monza beta-hydroxybutyrate (BHB) measurements ................................................................. 77

Figure 2.2: Number of normal (non-ketotic) and ketotic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on the Precision Xtra beta-hydroxybutyrate (BHB) values ≥0.1 mmol/L cut-off .................................................................................. 78

Figure 2.2.1: Number of normal (non-ketotic) and ketotic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on the Precision Xtra beta-hydroxybutyrate (BHB) values ≥0.2 mmol/L cut-off .................................................................................. 79

Figure 2.2.2: Number of normal (non-ketotic) and ketotic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on the Precision Xtra beta-hydroxybutyrate (BHB) values ≥0.3 mmol/L cut-off .................................................................................. 80

Figure 2.3: Number of normal (non-ketotic) and ketotic pigs by province based on Rx Monza beta-hydroxybutyrate (BHB) levels ........................................................................................................ 81

Figure 2.4: Number of normal (non-ketotic) and ketotic pigs by province based on Precision Xtra beta-hydroxybutyrate (BHB) levels ≥0.1 mmol/L cut-off .......................................................... 82

Figure 2.4.1: Number of normal and ketotic pigs by province based on Precision Xtra beta-hydroxybutyrate (BHB) levels ≥0.2 mmol/L cut-off .................................................................................. 83

Figure 2.4.2: Number of normal (non-ketotic) and ketotic pigs by province based on Precision Xtra beta-hydroxybutyrate (BHB) levels ≥0.3 mmol/L cut-off .................................................................................. 84

Figure 2.5: Bland-Altman plot evaluating agreement between Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values using ≥0.1 mmol/L cut-off value for defining ketosis ...................................................... 85

Figure 2.6: A non-parametric ROC curve\textsuperscript{\textcircled{a}} assessing the agreement between the Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values with a ≥0.1 mmol/L cut-off value for defining ketosis .................................................................................................................. 86

Figure 2.6.1: A non-parametric ROC curve\textsuperscript{\textcircled{a}} assessing the agreement between the Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values with a ≥0.2 mmol/L cut-off value for defining ketosis .................................................................................................................. 87

Figure 2.6.2: A non-parametric ROC curve\textsuperscript{\textcircled{a}} assessing the agreement between the Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values with a ≥0.3 mmol/L cut-off value for defining ketosis .................................................................................................................. 88
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ADFI</td>
<td>average daily feed intake</td>
</tr>
<tr>
<td>ADG</td>
<td>average daily gain</td>
</tr>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AIAO</td>
<td>all-in/all-out</td>
</tr>
<tr>
<td>ALP</td>
<td>alkaline phosphatase</td>
</tr>
<tr>
<td>AST</td>
<td>aspartate aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BCG</td>
<td>bromcresol green</td>
</tr>
<tr>
<td>BHB</td>
<td>beta-hydroxybutyrate</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
</tr>
<tr>
<td>$$CAD</td>
<td>Canadian dollars</td>
</tr>
<tr>
<td>CK</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>CRF</td>
<td>corticotropin-releasing factor</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>GGT</td>
<td>$\gamma$-glutamyltransferase</td>
</tr>
<tr>
<td>GLDH</td>
<td>glutamate dehydrogenase</td>
</tr>
<tr>
<td>Hb</td>
<td>hemoglobin</td>
</tr>
<tr>
<td>Hct</td>
<td>hematocrit</td>
</tr>
<tr>
<td>ISE</td>
<td>ion-selective electrode</td>
</tr>
<tr>
<td>LCT</td>
<td>lower critical temperature</td>
</tr>
<tr>
<td>ME</td>
<td>metabolizable energy</td>
</tr>
<tr>
<td>MCH</td>
<td>mean corpuscular hemoglobin</td>
</tr>
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</table>
MCHC – mean corpuscular hemoglobin concentration
MCV – mean corpuscular volume
NADH – nicotinamide adenine dinucleotide
NADPH – nicotinamide adenine dinucleotide phosphate
NRC – National Research Council
PFTS – periweaning failure to thrive syndrome
RBC – red blood cell
TIBC – total iron-binding capacity
CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1. Introduction

In modern swine production weaning is a significant event and in general stressful for the pig. Newly weaned pigs face physiological, social, nutritional, and environmental challenges. The change from a milk diet to a solid, grain-based feed corresponds with structural changes in the small intestine of pigs. Newly weaned piglets experience an initial reduction in feed intake, and the physiological changes in the gut include villous atrophy and crypt hyperplasia (Pluske et al., 1997). Within a week post-weaning, most pigs will have transitioned and adapted to their new diet and environment. However, a subgroup of newly weaned pigs will fail to overcome the challenges of weaning. Typically these pigs that fail to transition are healthy and robust at the time of weaning but once they are moved to the nursery room they consume little to no feed (Moeser et al., 2012). In severe cases this condition of failing to transition may be referred to as periweaning failure to thrive syndrome (PFTS). Pigs affected with PFTS are anorexic and develop a distinctive behaviour of repetitive sham chewing, or chomping in the early stages of the syndrome, often before there is significant loss in body condition. There is no known cause of this syndrome, and one of the difficulties in studying the condition is that early detection is difficult. However, it has been reported that anorexic pigs have elevated levels of beta-hydroxybutyrate (BHB), a serum ketone (O’Sullivan et al., 2014a). Since anorexia is a consistent characteristic of PFTS, the analysis of ketone levels on commercial farms may be a promising diagnostic tool to enable earlier identification of anorexia in pigs after weaning. Additionally, this may lead to a better understanding of post-weaning anorexia and a better understanding of PFTS.
Iron deficiency is the most common and preventable mineral deficiency in the swine industry. Hence, iron supplementation is an essential procedure for ensuring the health of suckling pigs. The major etiological factors associated with iron deficiency and anemia in pigs includes low fetal iron stores, the rapid growth rate of piglets and inadequate levels of iron in sow milk (Svoboda & Drábek, 2005a). The standardized protocol for the industry in preventing iron deficiency and anemia in piglets is to administer a 200 mg intramuscular injection of iron-dextran at 3d of age for piglets weaned between three and four weeks of age (Murphy et al., 1997). However, piglets are growing at a faster rate and born into larger litters compared to the production standards that were common when iron supplementation protocols were initially recommended and developed. With today’s production practices far surpassing those of decades ago it is critical to reassess whether the current recommended iron administration protocol is still sufficient in preventing iron deficiency anemia in pigs.

With increasing pressures to limit antibiotic usage in the feed of food-producing animals, the addition of high levels of zinc oxide (2000-3000 ppm) to nursery rations is a common practice in Ontario (Slifierz et al., 2015). Typically, the addition of zinc oxide in feed is used in the first 1 to 3 weeks post-weaning to reduce the incidence of diarrhea and to promote growth. However, this increase in zinc may interfere with iron absorption and may play a role in post-weaning anemia (Rinker et al., 2005). To get a better understanding of nursery pig health from Ontario commercial swine farms, full hematological and biochemistry profiles need to be assessed to determine benchmark values and reference ranges.
1.1.1 Production Practices

The health status of piglets in a commercial setting is often challenged due to various stressors after weaning. Newly weaned pigs are placed in new social and environmental conditions (Pastorelli et al., 2012a; Wellock et al., 2003) and exposed to new pathogenic agents (Kyriazakis and Houdijk, 2007). The degree of sanitation is another factor that can impact the ability for a pig to thrive in the nursery. Poor production practices, such as inefficient washing and disinfection of pens before placement of pigs, can result in poor feed intake and growth performance (Williams et al., 1997). It is therefore important to follow good production practices in order to maintain a healthy environment for newly weaned pigs to thrive.

The most appropriate age of weaning is often under debate as there are both advantages and disadvantages to younger (e.g. <17 d) and older (e.g. >28 d) weaning ages with regard to welfare and production. In the wild, weaning is a gradual process without a definitive time period but rather a shift from reliance on sow milk to the transition to solid feed, occurring anywhere between 12-17 weeks of age (Newberry and Wood-Gush, 1988; Jensen and Recén, 1989). This long weaning period benefits piglets because they are physically ready and more adaptable to the stressors of weaning. For instance, weaning at an older age allows for the piglet’s immune system to further develop and respond to disease challenge compared to the immature immune system of the younger piglet. However, in a commercial setting there is great economic advantage to weaning pigs at 3-4 weeks of age because the sow will produce more pigs per year and the expensive farrowing facility can be more efficiently used (Johnson et al., 2012).

When determining when to wean piglets in a commercial setting, there are many
factors to consider including herd health, management practices, piglet size, birth and weaning weights, genetics, sow nutrition, litter size, and the facilities (National Farm Animal Care Council, 2014). Commercial weaning for swine in North America typically occurs between 21-28 days of age with piglets weighing approximately 5-9 kg (Cox and Cooper, 2001; Le Dividich and Herpin, 1994). At times, piglets may need to be weaned earlier than what is recommended for various reasons including: 1) to control for disease, 2) if a sow is ill or dead, and 3) if a sow is not producing adequate amounts of milk, 4) if there are too many sows due to farrow and not enough farrowing spaces available (National Farm Animal Care Council, 2014). Once piglets are weaned, they remain in the nursery until they are around ten weeks of age and then moved to the grower-finisher barn. Some production systems wean pigs directly into a wean-to-finish facility. The Canadian code of practice for the care and handling of pigs states that weaning procedures must be developed and followed to minimize the negative impacts on the health and welfare of piglets:

“It is recommended that to target an average weaning age of 3 weeks or older and to source weanlings only from reputable suppliers with known health status. It is important to monitor all newly weaned pigs closely during the first 2 weeks after weaning for any signs of ill health. It is imperative to treat signs of ill-health promptly and to ensure that the area that piglets are weaned into is clean and dry (National Farm Animal Care Council, 2014).”

Weaning between 21-28 days of age on commercial farms optimizes piglet health, improves feed efficiency and growth rates, thereby improving economic productivity (Hohenshell et al., 2000). However regardless of weaning age, light weight piglets
require additional care and can often benefit from being placed in specialized pens until they are able to be moved to the common nursery area (National Farm Animal Care Council, 2014).

There are disadvantages of early weaning including inconsistent growth performance throughout the nursery and grower-finisher stage (Johnson et al., 2012) and abnormal feed intake (Orgeur et al., 2001). Worsaae and Schmidt (1980) found higher levels of cortisol, a hormone indicating stress, when piglets were weaned at 3 weeks of age compared to piglets weaned at 8 weeks of age. Also, piglets weaned at an early age were found to have immune systems that were not as responsive to disease challenge compared to piglets weaned at an older age (Johnson et al., 2012). McLamb et al. (2013) also found that piglets that were weaned early (16 d and 18 d) had a higher prevalence of diarrhea and poor growth rates in response to *Escherichia coli* challenge compared to pigs weaned at a later age (20 d).

Good management practices to prevent and minimize the stresses piglets face during weaning include management strategies to optimize sanitation, temperature, humidity and ventilation.

### 1.1.2 Sanitation

Sanitation is important in order to reduce the presence of pathogens in the environment and to minimize overall disease challenge. Nursery pens are built purposely with slatted floors made out of plastic or metal so that they can be easily cleaned and disinfected. Newly weaned piglets are at the highest health risk in the production phase because their immunity is not at its peak. This is because these piglets experience a
decrease in feed intake and face new challenges and stressors that occur simultaneously (Pastorelli et al., 2012a). Prior to the arrival of newly weaned pigs in the nursery barn, the room and all equipment must be properly washed and disinfected. Previous research has reported that up to 99% of bacteria can be eliminated through sanitation under experimental conditions. The order of importance for the stages of sanitation to reduce pathogen load include: 1) 90% removal of pathogen load by removing all visible organic matter, 2) 6 to 7% killed by disinfectants, and 3) 1 to 2% killed by fumigation, however this third stage is typically not used in the nursery barn (Morgan-Jones, 1987).

1.1.3 All-In/All-Out Production Protocols

All-in/all-out (AIAO) is a swine production strategy commonly used to keep pigs in groups matched by age, weight, stage of production and health condition throughout all the phases of production (Scheidt et al., 1995). When the facility is completely emptied and sanitized before the next batch is moved in, it is referred to as an AIAO nursery. An AIAO system can refer to the movement of pigs by room, building and site. The alternative is a continuous-flow system, where the facility is never completely empty. Since sanitation is so important to overall piglet health, almost all nursery rooms are emptied, washed, and disinfected before new piglets are moved in. This often occurs whether or not the entire facility is emptied (Scheidt et al., 1995). An AIAO production system is advantageous over a continuous-flow system because it can improve production, reduce the risk of disease transmission from old to young pigs and hence reduce production costs. Post-weaning piglets have an immature immune system, and thus if the nursery is not sanitized or contains older nursery pigs that may be shedding
infectious organisms, piglets are at additional risk of exposure to infectious disease(s) (Scheidt et al., 1995). An AIAO system prevents or minimizes this exposure since it reduces the risk of pathogens spreading from older pigs still present in the facility to the incoming group. An AIAO production system throughout all phases of production is also beneficial to farrowing room production. In the farrowing room, AIAO improves the control and treatment of diseases where a continuous flow system in the farrowing room often acts as a disease reservoir with newborn litters constantly being infected. In the nursery barn, AIAO allows for better control of the airspace and the rooms or barns can be managed appropriately since pigs are grouped by age and size, thus a proper temperature can be set for their needs. Therefore, using an AIAO production system is advantageous over using a continuous-flow system, as it will reduce stress and disease transmission, while improving swine health and performance.

1.1.4 Ambient Temperature

The ambient temperature requirement of a newly weaned pig is relatively high because feed intake is reduced in comparison to the late suckling period (Le Dividich and Herpin, 1994). There are two main periods of the post-weaning phase to be examined: the critical period and the post-critical period. The critical period is the first two weeks post-weaning where piglets learn to consume dry solid feed and recalibrate to the pre-weaning level of feed consumption. During the 4-6 days post-weaning, feed intake is reduced while physical activity is increased (McCraken and Caldwell, 1980). When this decrease in feed intake occurs the newly weaned pig has a reduction in body thermal insulation due to a reduction in body fat (Le Dividich and Herpin, 1994). This suggests that newly weaned pigs have an increased rate of heat loss and require an environment with a
warmer ambient temperature to accommodate for this loss. The lower critical temperature (LCT) should be 26-28°C in the first week post-weaning, since the average feed intake level is lower than the metabolizable energy requirements (McCraken and Caldwell, 1980). After the first two weeks post-weaning the LCT is reduced to 24°C and is associated with a daily metabolizable energy intake of 1100kJ/kg (Close and Stanier, 1984b). The purpose of having an increased ambient temperature in the critical first two weeks after weaning is to keep pigs warm and comfortable to meet their thermal requirements.

During the post-critical period, pigs have typically adjusted to feed and eat regularly with minimal risk of scouring. At this time, the temperature in the nursery can be reduced. Prior work suggests a 2-3°C decrease in ambient temperature on a weekly basis (Close and Stanier, 1984a; Le Dividich, 1981) is optimal. This weekly reduction in temperature should stop when the appropriate ambient temperature for the grower-finisher barn is met. After the initial 6 days post-weaning, the adjustment to feed intake is stabilized following exposure to the cooler temperature (Verhagen et al., 1988). Previous research has found that piglets are able to achieve a constant growth rate when the ambient temperature decreased from 25°C to 15°C and when feed consumption increased by 30 g/kg per 1°C decrease in temperature (Hata et al., 1986; Rinaldo and Le Dividich, 1991). Thus, it is important to maintain recommended ambient temperatures post-weaning in order to maintain piglet comfort, minimize piglet mortality and morbidity and to obtain optimal growth performance (National Farm Animal Care Council, 2014).
1.1.5 Ventilation and Relative Humidity

Ventilation is important for the elimination of water vapour and noxious gases, and to control temperature. Ventilation plays a major role in controlling the rate of heat loss from the facility and has a major impact on productivity (Le Dividich & Herpin, 1994). A draught-free environment has been found to increase the growth rate of pigs by 6% compared to draught-exposed pigs (Hacker et al., 1979; Muehling & Jensen, 1961). Ventilation rates are accountable for 80-90% of heat loss experienced in the nursery barn (Le Dividich & Herpin, 1994). Relative humidity has an effect on the health and performance of nursery pigs. Relative humidity was found to have an influence on energy metabolism at extreme temperatures (Kamada & Notsuki, 1987). Morrison et al. (1969) found that when relative humidity was increased from 60-70% to 90-95%, there was no effect on the growth rate for grower pigs at 22°C. However, when the temperature increased to 28°C, an 8% reduction in growth rate was observed. A proper functioning ventilation system is important for optimal pig health and performance.

1.2 Weaning Stress

In the wild, piglets gradually wean with the process occurring between 12-17 weeks of age (Jensen, 1986; Newberry and Wood-Gush, 1988; Jensen and Recen, 1989). However, in a commercial setting, piglets are usually weaned abruptly, typically between 21-28 days of age (Cox and Cooper, 2001; Le Dividich & Herpin, 1994). Piglets experience sudden changes in their environment, nutrition and social group (Cox and Cooper, 2001) and this poses physiological, social, nutritional, and environmental challenges to piglets.
The physiology and microbiology of the gastrointestinal tract are altered at the
time of weaning, primarily due to the dietary change from sow milk to a grain-based diet.
The stress of weaning can contribute to immune system abnormalities leading to
suboptimal pig health and decreased feed intake, typically during the first week post-
weaning (Campbell et al., 2013). Pigs experience social stress due to separation from
their dam and the mixing of litters post-weaning (Bryant and Ewbank, 1972; Arnone and
Dantzer, 1980). Lastly, environmental stress may occur after weaning due to piglets
being relocated to a new facility (Le Dividich, 1981).

1.2.1 Physiological Stress

The small intestine performs three main functions: 1) the absorbing and digesting
of nutrients; 2) the absorbing and secreting of electrolytes to attain a suitable viscosity of
luminal contents and to remove noxious agents; 3) acting as a selective barrier to protect
against noxious agents and pathogens (Lallès et al., 2004; Wijten et al., 2011). Within
the first two weeks post-weaning, piglets commonly experience changes in the small
intestine related to inflammatory responses (Hampson, 1986a; Hampson, 1986b; Kelly et
al., 1991a; Kelly et al., 1991b; Miller et al., 1984; Boudry et al., 2004). The changes
found in the small intestine post-weaning include villous atrophy (shortening of the villi),
crypt elongation (increase in crypt depth) as well as depressed levels of brush-border
enzymes (Boudry et al., 2004; McCracken et al., 1999).

The reduction in feed intake within the first 48 hours post-weaning is associated
with villous atrophy in the small intestine (Lallès et al., 2004; van Beers-Schreurs et al.,
1998). It was found that villous height can be reduced by 25-35% compared to the pre-
weaning villi height within the first 24 hours after weaning (Hampson, 1986a). Extreme villous atrophy reduces the absorptive capacity in piglets, leading to malnourishment and suboptimal health. Following weaning, a transient decrease in brush border enzymes occurs in the small intestine (Hampson and Kidder, 1986; Lindemann et al., 1986). Also at this time, piglets experience a decrease in lactase activity due to the decline in brush-border activity of lactase (Montgomery et al., 1981; Motohashi et al., 1997). These immediate changes after weaning can temporarily inhibit digestion, which can predispose piglets to malabsorption and diarrhea (Friend et al., 1970; Shields et al., 1980; Efird et al., 1982).

The intestinal barrier is made up of a single layer of epithelial cells that line the intestinal tract. This is the first line of defense against harmful microorganisms, toxins, bacteria and antigens, which reside within the lumen of the small intestine (Campbell et al., 2013; Gewirtz et al., 2002; Muehling and Jensen, 1961; Spreeuwenberg et al., 2001). A compromised intestinal barrier with increased intestinal permeability and inflammation is common in newly weaned pigs (Smith et al., 2010; Spreeuwenberg et al., 2001). When the intestinal barrier is compromised, harmful agents can cross the epithelium and result in inflammation, malabsorption, diarrhea, reduced growth and production (Waddell and Gyles, 1995; Nabuurs et al., 2001; Egberts et al., 1991). Systemic disease can occur when the intestinal barrier is disrupted (Moeser et al., 2007b). Thus, the translocation of harmful agents in the body due to a compromised intestinal barrier makes piglets more susceptible to infections (Berg, 1995; Uil et al., 1996).

Researchers have analyzed stress hormones and intestinal barrier function 7 days post-weaning and found that these pigs had increased concentrations of cortisol and
corticotropin-releasing factor (CRF) in blood plasma, indicating that weaning is a stressful transitional period (Santos et al., 2000; Moeser et al., 2007b). Piglets that were weaned at an older age were less susceptible to compromised barrier function and had lower permeability in their intestinal barrier (Smith et al., 2010; Moeser et al., 2007a). Compromised barrier function and increased intestinal permeability is likely related to premature maturation of the small intestine that is found in the early post-weaning period. Therefore, to improve intestinal barrier post-weaning three approaches have been suggested: 1) to improve the palatability of the starter feed to increase feed intake after weaning; 2) to identify important nutrients that may be given to piglets with poor feed intake post-weaning either through water or in a concentrated form to prevent the loss of barrier function; and 3) the addition of biologically active ingredients to inhibit the stress response to prevent the loss of barrier function (Wijten et al., 2011).

The stress from weaning can suppress feed intake. At birth piglets rely on the intake of colostrum for passive immunity. Piglets become colonized with lactic acid bacteria, enterobacteria and streptococci from the sow and the environment (Hopwood and Hampson, 2003). However, following weaning, piglets often experience a period of reduction in feed consumption or stop eating all together, as they are transitioned onto a more complex diet. During this time the gastrointestinal tract alters the availability of specific microbial substrates and these piglets become highly susceptible to enteric diseases post-weaning (Hopwood and Hampson, 2003; Madec et al., 2000). It is therefore imperative for pigs to develop immune-competence to ensure optimal health.
1.2.2 Social Stress

Along with physiological stress, weaning stimulates many social stresses. Piglets are separated from sows abruptly and may experience stress due to handling and transportation when relocated to the nursery barn (Bryant and Ewbank, 1972; Funderburke and Seerley, 1990). When piglets are weaned, litters are often mixed to make a more homogenous group based on gender, weight or health condition. When litters are mixed, a social hierarchy is determined amongst the new pen mates (Dybkjaer, 1992). Mixing of litters at weaning was found to increase stress in piglets, leading to elevated plasma cortisol levels (Blecha et al., 1985) and aggression (Arnone and Dantzer, 1980). The development of dominance in the new social hierarchy has been found to lead to a reduction in feed intake and impose nutritional stress during this critical development stage. Therefore, it is important to understand the different social stresses that may impact the overall health and performance of newly weaned pigs.

1.2.3 Nutritional Stress

Weaning is a stressful time for piglets as their diets are altered from highly digestible and palatable liquid milk to a less digestible and more complex solid feed (Lallès et al., 2007). Prior to weaning, piglets are often provided with creep feed to help with the transition to solid feed following weaning. Piglets are challenged with a dramatic change in the source of nutrients they must consume post-weaning. Prior to weaning, piglets were consuming milk from the sows, which consists of a low-carbohydrate and high-fat diet. However, post-weaning their diets switch to solid feed, which is high in carbohydrates and low in fats (Le Dividich and Herpin, 1994).
Weaning is often associated with a reduction in feed consumption (Pluske et al., 1997). As a consequence, piglets become malnourished with a transient decreased growth rate. While 50% of piglets consume feed within 24 hours of weaning, approximately 10% of piglets do not consume their first meal prior to the first 48 hours post-weaning (Brooks et al., 2001). It is estimated that a piglet’s metabolizable energy (ME) intake is about 60-70% of their pre-weaning milk consumption during the first week post-weaning (Campbell et al., 2013), and that it takes between 8-14 days for these piglets to recover their pre-weaning level of metabolizable energy intake (Marion et al., 2002). However, the duration of the period of decreased feed consumption varies (Bruininx et al., 2002; Le Dividich and Sève, 2000). The variability in feed consumption post-weaning may be linked to weaning weight, genotype, and gender (Lallès et al., 2007). The introduction of creep feed in the suckling period has been found to improve early post-weaning voluntary feed intake (VFI). The use of creep feed prior to weaning has also been shown to be a protective effect against disease (English, 1980). Carstensen et al. (2005) found that *E. coli* infection was lower in piglets consuming creep feed during the suckling phase compared to piglets who did not consume creep feed at this time.

Growth performance is reduced when there is a decrease in feed consumption. Generally, pigs exhibit a loss from 100-250 g of body weight (BW) on the first day of weaning and recover this weight loss by approximately four days post-weaning (Le Dividich and Sève, 2000). Performance in the first week post-weaning has an impact throughout the other phases of the barn. Piglets that gain more than 227 g/d within the first week post-weaning were found to have a reduction in days to market by 6-10 days compared to pigs that gained between 0-150 g/d (Tokach et al., 1992). Also,
inflammation due to an allergy from the introduction of feed with new ingredients such as soybean meal may also occur at the time of weaning. Therefore, it is crucial to make sure that piglets are consuming sufficient feed post-weaning in order to improve growth rates, productivity and to prevent disease, but also that the feed is nutritious, digestible and unlikely to create inflammation or allergic reactions (Campbell et al., 2013).

1.2.4 Environmental Stress

Environmental conditions play a major role on the overall health status of weaned piglets. Following weaning, gastrointestinal disturbances can occur due to adverse environmental conditions. Both chronic and acute changes in the environment can affect piglet health. Piglets exposed to chronic low temperatures (18-20°C) post-weaning were found to have a higher incidence of diarrhea (Le Dividich et al., 1980; Close and Stanier, 1984a). Piglets exposed to draughts had a higher incidence of coughing, sneezing, diarrhea and poor growth rates (Scheepens et al., 1991).

The sanitary conditions of nursery barns can impact the way these newly weaned piglets adapt to the new stresses (Pastorelli et al., 2012b). Sanitary challenges reduce feed intake and growth, leading to poor piglet health and economic losses (Pastorelli et al., 2012a). Sanitary conditions in a commercial setting vary between farms and poor sanitary conditions are found to negatively impact performance (Klasing and Johnstone, 1991), induce inflammatory responses (Klasing and Johnstone, 1991; Le Floc’h, et al., 2006), impede growth, and inflict health disturbances, (i.e. particularly digestive disorders) (Madec et al., 1998). Bacterial and viral challenges are difficult to overcome in an environment with poor sanitation practices.
1.3 Post-weaning Anorexia

The stressful and abrupt transition of weaning alters structural changes in the small intestine of pigs. The small intestine is composed of an epithelial lining with finger-like protrusions known as villi (Heo et al., 2013). Villi are important because they are used to increase the surface area of the small intestine for better digestion and absorption of nutrients (Zhang et al., 2007). For the small intestine to function well, long villi are necessary. The small intestine also contains crypts, which contain the epithelial stem cells which repopulate the apical cells of the villi (Zhang et al., 2007). After weaning, villous atrophy and crypt hyperplasia occurs. Post-weaning anorexia is thought to be the main factor for these intestinal changes, since the energy intake after weaning is positively related to intestinal structure (Pluske et al., 1997). Post-weaning anorexia has been found to be correlated with crypt hypertrophy and local inflammation, as McCracken et al. (1999) found that a decrease in feed intake compromised the epithelium structure in the jejunum of pigs fed a soybean-based diet after weaning. It was also found that as normal feed consumption patterns resume, intestinal inflammatory responses subside and epithelial morphology improve (McCracken et al., 1999).

Following weaning, there is a significant reduction in feed consumption (Boudry et al., 2002), growth performance, and intestinal functionality (Miller et al., 1984; Moeser et al., 2007b) due to the new stresses and challenges facing newly weaned pigs. Although this reduced performance is common, most pigs do recover within a few days after weaning. However, there is a subgroup of pigs that do not overcome these new challenges and often remain unthrifty, and can have high morbidity or mortality rates due to post-weaning anorexia (Moeser et al., 2012). It is often challenging to identify piglets
in a commercial setting that are experiencing anorexia. Piglets prior to weaning may have failed to consume creep feed and may not have transitioned onto feed post-weaning, without the producer identifying them. Hence, it would be beneficial to be able to identify anorexic piglets early so different management strategies can be implemented to improve feed consumption for this subgroup of pigs and to minimize the negative consequences of post-weaning anorexia.

1.4 Periweaning Failure to Thrive Syndrome (PFTS)

Periweaning failure to thrive syndrome (PFTS) is a clinical condition recognized in commercial swine production and is associated with weaning (Gauvreau and Harding, 2008; Moeser et al., 2012; Segalès et al., 2012). Previously, PFTS was known as post-weaning catabolic syndrome (Moeser et al., 2007b), and post-weaning wasting-catabolic syndrome (Friendship et al., 2010). However, in 2010 the name PFTS was proposed to better represent the clinical syndrome being reported and to account for any potential peri-weaning risk factors associated with the syndrome (Moeser et al., 2007b; Huang et al., 2011). No current evidence has been reported that supports any association of environmental factors, infectious agents, or management practices with PFTS-affected pigs (Huang et al., 2011). However, a recent study conducted by Ramis et al. (2015) found an individual genetic predisposition in boars, with some producing higher numbers of PFTS-affected pigs compared to others. A single boar produced between 40-50% of PFTS-affected pigs when compared to healthy control piglets (Ramis et al., 2015). Thus, this new finding suggests that some piglets will have an individual genetic susceptibility to PFTS, however further research is needed.
The prevalence of farms reporting PFTS in North America is approximately 4.3% with some herds experiencing up to 20% mortality attributed to PFTS-affected pigs (O’Sullivan et al., 2014b). Hence, the economic losses due to morbidity and mortality rates attributed to this syndrome are a substantial problem for the swine industry.

Typically, PFTS-affected pigs are identified within 7 days of weaning. But, often, the loss of body condition and eventual debilitation associated with PFTS is not readily noted until the second week after weaning. These pigs become severely debilitated and are euthanized for humane reasons. The prevalence and severity of clinical signs observed in PFTS-affected pigs is independent of age since weaning age does not affect whether the newly weaned piglet will develop PFTS (Moeser et al., 2012). Within 7 days post-weaning, these once healthy and robust pigs develop clinical signs of anorexia, such as having hollow abdomens, visible spine, visible shoulder blades and hip prominences (Moeser et al., 2012). The loss in body condition is not due to a physical inability to eat as video footage shows that these pigs are able to pick up feed and chew (Harding and Huang, 2011) suggesting that PFTS-affected pigs have a reduction in appetite. However, PFTS-affected pigs are anorexic for an extended amount of time prior to being identified and these pigs may have not transitioned to feed both prior to weaning and afterwards.

PFTS-affected pigs display abnormal oral behavior consisting of repetitive licking, chomping and chewing (Huang et al., 2012). This behavior is known as sham chewing and PFTS-affected pigs can be observed standing for prolonged periods of time with their heads drooped while repeating this oral behavior. By the second week post-weaning, affected pigs become lethargic and fail to grow. Previous reports based on post mortem examinations documented that PFTS-affected pigs were emaciated with a loss of
body fat reserves, a clinical sign of ketosis (Huang et al., 2012). The most prevalent histological lesions in PFTS-affected pigs include: thymic atrophy, villous atrophy in the small intestine, and lymphocytic and neutrophilic rhinitis (Huang et al., 2012). At this time, the significance of these lesions in PFTS-affected pigs are not clear since some of these lesions have also been associated with periods of anorexia post-weaning (Harding and Huang, 2011). A PFTS case definition has been developed and is as follows:

“PFTS is characterized clinically by the progressive debilitation of weanling (nursery) pigs in the absence of discernable and detrimental infectious, nutritional, managemental, or environmental factors that can explain the clinical syndrome. At weaning, affected pigs are of average or above average body weight, and neither affected pigs nor their cohorts show evidence of residual illness from the suckling phase. Within 7 days of weaning, affected pigs are anorexic and lethargic. They deteriorate and within 2 to 3 weeks of weaning demonstrate marked muscle weakness and loss of body condition. Some affected pigs in all affected farms show repetitive oral behavior such as licking, chewing or chomping. In affected farms, morbidity and mortality by batch varies over time, but case fatality is high” (Huang et al., 2011).

In a recent study, O’Sullivan et al. (2014a) examined the behavioral and biochemical changes in anorexic piglets post-weaning to determine their status when fasted and then following the re-introduction to feed. The results reported stated that all fasted pigs developed repetitive chomping, chewing and licking behaviour by day 3 of the study, which is also a predominate clinical sign observed in PFTS-affected pigs (O’Sullivan et al., 2014a). It was also found that the fasted pigs had significantly higher
levels of beta-hydroxybutyrate (BHB), a serum ketone, compared to the pigs that ate normally. In a second phase of the study, a group of pigs were fasted for up to 8 days after weaning and then were introduced to feed on day 8. Upon the subsequent reintroduction of feed to these piglets they ate readily and, their BHB levels normalized within 48 hours (O'Sullivan et al., 2014a). These findings suggest that pigs not consuming feed (anorexic) in the post-weaning period will have elevated levels of the ketone BHB in their blood and will normalize when consumption resumes. Further research is needed to identify a diagnostic tool to aid in the early diagnosis of PFTS-affected pigs and to subsequently reduce nursery pig morbidity and mortality associated with this syndrome. The examination of piglet ketone levels under a commercial setting may be a promising diagnostic tool to identify anorexic pigs earlier in the development of the syndrome and before they become severely debilitated.

1.5 Ketones and Ketosis

Ketosis is a process where elevated levels of ketones are formed due to fatty acid degradation occurring in the liver. Ketones are small carbon fragments produced from the breakdown of fat stores, which are used for energy. Beta-hydroxybutyrate (BHB) is a serum ketone, which is produced in the liver and exported to peripheral tissues to use as an energy source (Luimes et al., 2011). This increase in the production of ketones is a result of reduced caloric intake by fasting or having increased energy demands such as high levels of milk production in dairy cattle. Diabetes mellitus is a metabolic condition that is associated with ketosis (Pegorier et al., 1982). Ketosis occurs when there is not enough glucose available due to the reduction of carbohydrates and thus the body needs
to rely on another source for energy. When ketotic, a state of negative energy balance occurs due to the stimulation of lipolysis (the breakdown of fats and other lipids by hydrolysis to release fatty acids) and anorexia (Pegorier et al., 1982).

Ketosis is a condition that is rarely reported in pigs (Alsop et al., 1994; Pegorier et al., 1982). There is very little literature on ketosis in pigs possibly due to the rarity of the condition or because it goes undetected. However, there is one study that investigated ketosis in lactating sows. In this study a handheld BHB meter was used because it was found to measure BHB accurately in blood of dairy cattle. The results of the study indicated that normal stress of lactation and fetal growth had no effect of BHB for sows (Luimes et al., 2011). However, anorexic sows had higher levels of BHB in their blood compared to sows consuming feed (Luimes et al., 2011). These findings suggest that anorexic sows developed lipolysis due to a negative energy balance. Currently there are no studies examining ketosis in weaned piglets to better understand anorexia. Therefore, it would be useful to examine ketone bodies in newly weaned piglets to help understand post-weaning anorexia and to aid in the understanding of PFTS.

1.6 Iron

Iron is necessary for normal cell function and is a component of various enzymes. In order for survival, cells of nearly all forms of life require defined amounts of iron for replication and expression (Morris et al., 1995). Iron exists in many forms bound to protein as heme compounds (hemoglobin or myoglobin), heme enzymes (catalase and peroxidase), or as non-heme compounds (transferrin and ferritin) (Dukes and Swenson, 1970). Iron can readily accept and donate electrons, altering from ferric (Fe$^{3+}$) and
ferrous (Fe$^{3+}$) forms. The ability for iron to alter between these two forms allows iron to
be a useful component for oxygen-binding molecules and various enzymes.

Iron is a necessary component in the formation of hemoglobin, a protein that
makes up roughly one-third of a red blood cell in weight (Miller and Ullrey, 1977). Since
iron is an important component of heme, its absence leads to a reduction in circulating
hemoglobin. Hemoglobin represents approximately 60% of total body iron (Dukes and
Swenson, 1970). Therefore, factors affecting the concentration of blood hemoglobin
levels severely affect the total iron status in the body or vice versa.

1.6.1 Iron Deficiency and Anemia

Iron is an essential nutrient and the most common mammalian mineral deficiency.
Iron deficiency and anemia are severe consequences of mineral deficiency and a
worldwide problem that affects both humans and domestic animals. Of all domestic
animals, the suckling pig is most prone to iron deficiency and anemia under current
commercial husbandry practices (Dale et al., 1961). Iron deficiency and anemia is
frequently seen in pigs regardless of their breed and is most prevalent in the neonatal
period (Collard, 2009).

1.6.2 Etiology of Iron Deficiency and Anemia in Pigs

Iron deficiency and anemia develops quickly in pigs for various reasons. Firstly,
newborn piglets are born with limited iron (approximately 50 mg in total) in their body,
mostly in the form of hemoglobin in blood, with only a very small amount stored in the
liver. These initial iron stores become depleted rapidly since the daily iron requirement
for piglets is about 7 mg/day (Murphy et al., 1997). Colostrum and milk from the sow contain important nutrients that piglets require, but are low in iron. Due to the low concentration of iron in the sow’s milk, piglets can only obtain about 1 mg of iron daily (Venn et al., 1947). Since sow milk only supplies piglets with about 1/7th of their daily iron requirements, this explains the need for additional supplemental iron sources to prevent anemia (Dale et al., 1961). The administration of large quantities of iron to the sow during late gestation via feed or injection has been investigated as a way to increase iron stores in piglets (Miller and Ullrey, 1977). This method has not been successful for preventing iron deficiency in newborn pigs.

Soil is a great source of iron for piglets when they are raised outdoors. However, in Canada because of the harsh climate sows and piglets are generally housed indoors. Therefore, with no access to soil, iron supplementation is necessary. The rapid growth rate of newborn piglets also contributes to high iron requirements. During the initial week of life, piglets double their weight from around 1.5 to 3 kg, with plasma volume increasing by 30% (Weiss and Wardrop, 2011). By week three of life, piglets normally reach four to five times their birth weight and eight times their birth weight by eight weeks of age (Svoboda and Drabek, 2005a). For many years pigs have been bred and selected for large litter sizes, high birth weights and fast growth rates. As piglets grow more rapidly, they are at greater risk for iron deficiency anemia. This results in increased blood volume and red blood cell (RBC) counts, consequently increasing iron demands (Calvo and Allue, 1986; Lipiński et al., 2010; Starzyński et al., 2013).
1.6.3 Immunocompetence

Iron deficiency leads to a reduction in immunocompetence, so that anemic piglets have reduced resistance to infectious and parasitic diseases (Svoboda and Drabek, 2005a). This results in poor production performance and considerable economic losses. With inadequate iron supplies in rapidly growing piglets, their ability to synthesize antibodies to overcome disease challenge is impaired. This is because iron is a key component in the enzymes used to produce antibodies.

Iron deficient and anemic pigs are more prone to diarrhea, often due to *E. coli* infections, since they have suboptimal immunity (Svoboda and Drabek, 2005a). Villous atrophy in the small intestine and alterations in gastrointestinal flora are found in iron deficient and anemic piglets (Svoboda and Drabek, 2005a), which may also partially explain why these pigs are more susceptible to diarrhea. Iron deficiency anemia reduces energy dependent intestinal reabsorption, which results in the malabsorption of disaccharides, leading to diarrhea (Dallman, 1986).

1.6.4 Clinical Signs of Iron Deficiency and Anemia

Pigs that are anemic will appear pale in colour (pallor) around the nose, eyes, and mouth by 7 days of age (Cromwell, 1995). These pigs will not always appear to grow slower than pigs with a normal iron status, however these pigs may fail to thrive when iron deficiency is worsened. Piglets with iron deficiency and anemia generally grow slower compared to piglets with sufficient iron (Svoboda and Drabek, 2005a). This reduction in growth rate may be explained by the fact that iron ions are essential for many biochemical reactions including the transformation of adenosine diphosphate (ADP) to...
adenosine triphosphate (ATP) (Svoboda and Drabek, 2005a). Iron deficiency and anemia can also result in a compromised immune system which subsequently contributes to other health issues such as dyspnea (shortness of breath) due to a drop in oxygen carrying capacity of blood or gastrointestinal upset (diarrhea). If iron supplementation is insufficient it is often the larger and faster growing pigs in the litter that are most affected by anemia (Cromwell, 1995).

1.6.5 Indicators of Iron Deficiency and Anemia

Diagnosing iron deficiency and anemia in piglets is based on clinical signs, laboratory analysis and a prior history of limited access to iron (Svoboda and Drabek, 2005a). The main method of confirming a diagnosis of iron deficiency anemia in piglets involves assessing their hematological status. Indicators for this include tests for hemoglobin, packed cell volume, erythrocyte count, serum iron and serum total iron binding capacity.

1.6.5.1 Hemoglobin

Hemoglobin (Hb) is a protein found in red blood cells that contain iron and functions to carry oxygen to tissues in the body. Decreased hemoglobin concentrations reduce the oxygen carrying capacity of the blood. Piglets undergo a 25% decrease in hemoglobin blood concentrations by day 3 of life and iron supplementation is needed to prevent and reverse this (Miller et al., 1961). Measuring hemoglobin concentration is often used as a diagnostic tool for determining the iron status of pigs. Anemia occurs when there is not enough hemoglobin in the blood to transport oxygen from the lungs to the body tissues. In addition, an inadequate supply of hemoglobin in the blood results in
the inability of hemoglobin to carry carbon dioxide from the body tissues to the lungs for exhalation (Cromwell, 1995). Gentry et al. (1997) found that pigs with higher hemoglobin concentrations at weaning had a greater average daily gain (ADG) postweaning. Improved growth performance has also been found in pigs with higher hemoglobin concentrations compared to those with lower concentrations (Schrama et al., 1997). It has also been noted that as weaning weight increases, hemoglobin concentrations decrease (Jolliff & Mahan, 2011).

For pigs between one and eight weeks of age, a hemoglobin concentration greater than 110 g/L is defined as normal (Svoboda & Drabek, 2005a). Anemia has been defined by many as a blood hemoglobin concentration below 80 g/L in piglets at weaning (Svoboda & Drabek, 2005a). However, with current changes in genetics, increased litter sizes and growth rates, there is evidence that suggests higher levels of hemoglobin concentrations may be needed for pigs to thrive. Nielsen et al. (2013) have suggested that a blood hemoglobin concentration below 110 g/L indicates iron deficiency and a blood hemoglobin concentration below 90 g/L indicates anaemia. It has been reported that the limitation to using Hb to measure iron is that its less sensitive for diagnosing iron deficiency and anemia compared to other measures (Cook, 2005).

1.6.5.2 Packed Cell Volume

Packed cell volume (hematocrit or Hct) can be used as a diagnostic test to determine the amount of functioning iron found in the body. It has been recorded by many that by 3 days of age, piglets undergo a 25% decrease in hematocrit levels (Miller et al., 1961). Administering iron to piglets can reverse this decrease in hematocrit levels. Jolliff and Mahan (2011) found that as piglet weaning weight increases, hematocrit
concentrations decrease. For pigs between one and eight weeks of age, the normal range for hematocrit is between 0.36 L/L and 0.42 L/L (Svoboda and Drabek, 2005a). These researchers found that the reduction in hematocrit concentrations were more significant than hemoglobin concentrations, indicating that hematocrit may be a better diagnostic measure to evaluate iron status in young pigs.

1.6.5.3 Erythrocytes

Erythrocytes, also known as red blood cells (RBCs) are microscopic biconcave circular discs varying in thickness and diameter that differ between animals and their nutritional status (Dukes and Swenson, 1970). The bone marrow of pigs is where erythropoiesis (formation of red blood cells) occurs. The normal range of erythrocytes in pigs is between 5.5-6.8 $10^{12}$/L (Svoboda and Drabek, 2005a). However, variations exist within pigs since cells are not uniformly distributed in the blood vascular system. This variation exists since plasma fluids are constantly shifting across capillary walls, resulting in differences in red blood cell counts between arterial and venous blood samples (Dukes and Swenson, 1970). Piglets undergo a 25% decrease in red blood cells by day 3 of life and an iron supplementation is needed to reverse this (Miller et al., 1961).

1.6.5.4 MCV, MCH, MCHC

With known values for hemoglobin, packed cell volume and erythrocyte number (RBCs), three erythrocyte indices can be calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). These three indices are important for diagnosing anemia in various mammals. The normal range for pigs between one and eight weeks of age is $58-63 \times 10^{15}$/L (MCV),
19-21 x 10^{12} \text{L} (\text{MCH}) \text{ and } 320-350 \text{ g/L (MCHC)} \text{ (Svoboda and Drabek, 2005a)} \text{. All three of these indices decrease with iron deficiency anemia. The MCV provides the average cell size while the MCH expresses the average weight of hemoglobin present in erythrocytes (Dukes and Swenson, 1970)\text{. MCHC provides the average percentage of MCV that is occupied by hemoglobin (Dukes and Swenson, 1970)\text{. There tends to be considerable variation in these three indices due to error in counting erythrocytes. Therefore, these measures may not be the best indicators for iron deficiency anemia.}}

1.6.5.5 \text{ Serum Iron & Serum Total Iron-Binding Capacity}

Serum iron is important because it measures circulating iron that is bound to transferrin. The normal range of serum iron for pigs is between 21.7 \pm 5.9 \text{ mmol/L} \text{ (Svoboda and Drabek, 2005a)} . Both iron deficiency anemia and bacterial infections were found to lead to a decrease in serum iron. When transferrin is saturated with iron, the iron content is referred to as total iron-binding capacity (TIBC). Since transferrin can bind more iron than what is normally present, the TIBC is greater than serum iron. The difference between TIBC and serum iron is expressed as unsaturated iron-binding capacity (UIBC).

1.6.6 \text{ Oral Iron Supplementation Practices and Dosing}

Prevention of iron deficiency during the neonatal period can be accomplished by supplementing suckling piglets with an oral iron supplement. There are multiple methods of oral iron application that have been used over the years. Some of these methods include: providing suckling pigs access to soil in their pens, applying a viscous iron paste on the teats and mammary glands of lactating sows, spraying an aqueous solution of iron
compounds on the mammary glands of sows, and administering iron solution or tablets orally (Svoboda and Drábek, 2005b). The effectiveness of oral iron supplementation regimens in the attempt to prevent iron deficiency anemia in swine is inconsistent. Using pastes containing iron is one form of oral supplementation that is beneficial because it can also be used as a source for selected minerals and vitamins. Another common method used to administer oral iron is by enriching pre-starter diets with readily absorbable forms of iron and offering to piglets ad libitum. However, this method is often insufficient in preventing iron deficiency anemia because the consumption of pre-starter diets in the first two weeks after birth is low (Svoboda and Drábek, 2005b). The types of oral iron supplementation that can be administered to piglets include iron-dextran, iron salts or chelates.

Newborn piglets have the ability to absorb macromolecules such as dextran from the small intestine through pinocytosis. This process provides piglets with passive immunity through the sow’s colostrum (Svoboda and Drábek, 2005b). The uptake of intestinal macromolecules that have been transmitted across the intestinal wall to the blood occurs in-utero during the last two weeks of gestation, however the uptake is decreased in suckling pigs (Svendsen et al., 2005). This suggests that the ability to transfer and uptake intact proteins from the epithelium into circulation is a specific process that does not develop until close to term (Svoboda and Drábek, 2005b). This supports the findings that protein absorption is lower in premature newborn piglets compared to piglets born at full term (Sangild et al., 1999). In pigs, the ability to absorb macromolecules is limited to the first day of life, after which a process known as
intestinal closure occurs (Svendsen et al., 2005) and the pig’s ability to absorb macromolecules ceases.

When piglets are administered an oral dose of iron-dextran, the iron-dextran moves into the epithelial cells approximately twenty hours after administration (Svoboda and Drábek, 2005b). The epithelial cells will remain functional and transfer the iron-dextran to blood plasma within seven days after the piglet has received the oral supplement. Iron-dextran is a water-soluble compound that is transported in blood plasma. Due to the large molecular size of iron-dextran, the kidney does not excrete it. Most of the iron is taken up by the reticuloendothelial system (cells with main functions in phagocytosis and storage), with dextran broken down by lysosomal enzymes and stored as ferritin (Svoboda and Drábek, 2005b). To maximize the efficiency of iron-dextran, it must be administered immediately after birth. The administration of iron-dextran should be given within the first ten hours after birth before intestinal closure occurs.

The recommended dosage of oral iron-dextran is debatable. The administration of iron-dextran (150 mg Fe) within eight hours after birth, followed by a second 150 mg Fe dose on day nine resulted in normal hemoglobin blood concentrations (Svoboda and Drábek, 2005b). A single dose of iron-dextran (200 mg Fe) was found to result in lower hemoglobin blood concentrations than the double dose, however it was still successful in preventing iron deficiency anemia (Svoboda and Drábek, 2005b). Also, this strategy was found to be equivalent to a 200 mg iron-dextran dose given parenterally (injection).

Iron-sulfate and iron-fumarate are iron salts found in the intestine that release iron ions (Svoboda and Drábek, 2005b). Gleed and Sansom (1982) have repeatedly reported
that feeding sows a rich iron-sulfate diet (2 g of iron per kg of feed) protected piglets from iron deficiency anemia without the need for an iron-dextran injection. The growth rates of piglets were also comparable to piglets given a single dose of 200 mg iron-dextran injection intramuscularly.

In a previous study, the efficiency of iron-fumarate was analyzed in a paste form, and administered to day-old piglets. The oral dose of 100 mg fumarate-bound iron given to piglets was found to have equivalent hematological indices compared to a parenteral 200 mg Fe\(^{3+}\), with some indices even stronger during the second week of life (Svoboda and Drábek, 2005b). Svoboda and Drabek (2002) found that a repeated dose of 200 mg iron-fumarate on days 6 and 11 were required in order to have comparable hematological values to a 200 mg intramuscular iron-dextran injection. This may be due to intestinal disorders that develop in the postnatal period, affecting the utilization of orally administered iron (Svoboda and Drábek, 2005b).

The last type of oral iron supplementation for piglets is amino acid-chelated iron. The high stability of iron chelates allows them to remain intact in the gastrointestinal tract and to be absorbed as a complex (Svoboda and Drábek, 2005b). Although the iron chelates are well absorbed, some are excreted by the kidneys. However, the use of iron chelates in water for suckling piglets as a sole iron source was found to be inefficient (Svoboda & Drábek, 2005b). One study found that sows that were given 300 mg of amino acid-chelated iron daily resulted in piglets having a small increase in hemoglobin and red blood cell count in the piglets (Svoboda & Drábek, 2005b). Piglets were found to have an increase in hemoglobin and red blood cell count, however this was not significant and thus impractical. Due to the increased amount of labour needed to
administer oral iron supplements and their inconsistent results, single or multiple intramuscular injections of iron compounds are more commonly used for preventing iron deficiency anemia (Dale et al., 1961).

1.6.7 Parenteral Iron Supplementation Practices and Dosing

Iron injections containing iron-dextran, hydrogenated iron-dextran, dextrin-ferric oxide complex and polysaccharide-iron complex are used in commercial swine production to prevent iron deficiency anemia in piglets (Svoboda and Drábek, 2005b). Gleptoferron is another substance used for intramuscular iron application and it was found to have similar efficiency in preventing iron deficiency anemia when compared to iron-dextran injections (Svoboda and Drábek, 2005b). By 3 days of age, piglets experience a 25% reduction in blood Hb, Hct and RBCs (Miller et al., 1961). These researchers found that hematological indicators were reversed when a single intramuscular injection of 100 or 150 mg iron-dextran was given at 3 days of age and hemoglobin levels were sustained for four weeks.

An injected dose of iron-dextran should ensure that each piglet has sufficient iron to maintain a blood hemoglobin concentration above 100 g/L until weaning (Svoboda and Drábek, 2005b). Early research found that a single intramuscular injection of 100 mg of iron-dextran was sufficient in preventing iron deficiency anemia (Ullrey et al., 1959). However, recent research has found that a single intramuscular injection of 100 mg of iron-dextran was not sufficient in preventing iron deficiency anemia and piglets did not grow as fast compared to piglets given a 200 mg dose of iron-dextran (Svoboda and Drábek, 2005b). For piglets weaned between 3 and 4 weeks of age, the most common approach in preventing clinical iron deficiency anemia in piglets is to administer a 200
mg intramuscular injection of iron-dextran at 3 days of age (Murphy et al., 1997). However it has been suggested that in order to prevent subclinical iron deficiency, additional iron at a minimum level of 240 mg per kg of feed or a second iron injection may be necessary (Svoboda and Drábek, 2005b). Additionally, it has been suggested that in order to obtain optimal hematological parameters (RBC, Hb, etc.) quality pre-starter feeds with additional iron should be provided to piglets ad libitum (Svoboda and Drábek, 2005b).

A previous study was conducted to determine the differences between supplemented iron dextran dosages on hemoglobin concentrations and piglet growth during the suckling period. Researchers compared piglets given a 200 mg injection of iron dextran with piglets given a 300 mg injection. They reported no anemic pigs in either group and similar growth rates (Murphy et al., 1997). Jolliff and Mahan (2011) found that hemoglobin blood concentrations and percentage Hct were significantly higher when 300 mg of iron-dextran was injected compared to a single 200 mg iron-dextran injection at birth. Likewise a combination of a 200 mg injection at birth along with a 100 mg iron-dextran injection at day 10 of age resulted in higher hemoglobin levels compared to a single 200 mg injection (Jolliff and Mahan, 2011). Their study also found no significant difference between the various iron doses and growth rate. After 21 days post-weaning, there was little difference found between Hb and Hct values between the pigs that had received the 200 mg and 300 mg iron injections (Jolliff and Mahan, 2011). Hemoglobin and Hct values were lowest between 14 and 21 days post-weaning (31 to 38 days of age). This indicates that Hb synthesis and Hct production does not increase proportionately to the rapid growth rate. Miller et al. (1961) suggest that this decline in Hb and Hct values is
due to the rapid increase in plasma volume compared to erythrocyte numbers.

Hemoglobin and Hct values increase at a later point in the nursery and this suggests that between 31 and 48 days of age a critical time exists for iron metabolism and erythropoiesis for pigs (Jolliff and Mahan, 2011).

Hansen et al. (2010) found that the intestinal regulation of iron absorption may not be fully functional during the first two weeks after weaning at 21 days of age. It has been reported that mRNA levels for the iron transporters divalent metal transporter 1 and ferroportin are not up-regulated in the duodenal mucosa until 26-47 days of age. Jolliff and Mahan (2010) also found this time frame to be critical for iron utilization and the age when dietary iron begins to affect Hb and Hct values.

Kernkamp et al. (1962) looked at the effectiveness of administrating iron-dextran, iron-dextrin or ferric ammonium citrate with B12 in piglets at 3 days of age in preventing iron deficiency anemia. Control piglets that received no iron injection on day 3 of life had a marked decrease in blood hemoglobin compared to piglets that received an iron-dextran or iron-dextrin injection at 3 days of age (Kernkamp et al., 1962). An increased growth rate was also seen in pigs that received the iron-dextran or iron-dextrin injection compared to the control pigs. An injection of ferric ammonium citrate with B12 on day 3 was much less effective in increasing blood hemoglobin and growth rate compared to an injection of iron-dextran or iron-dextrin (Kernkamp et al., 1962).

There have been inconsistent results obtained for using multiple iron-dextran injections for preventing iron deficiency anemia. It has been found that two intramuscular injections of 100 mg of iron-dextran on day 3 and 10 resulted in greater blood hemoglobin levels compared to a single injection (Maner et al., 1959). The additional
injection was not found to improve weight gain in this study and thus may not be economical. However, another study found higher growth rates in pigs given the additional injection (Svoboda and Drábek, 2005b).

1.6.8 Reassessment of Iron Supplementation Protocols

The majority of farms use the standardized iron supplementation regimen, a single injection of 200 mg of iron within the first 3 days of life. With updated management practices as well as the use of new genetic lines of pigs, the standardized iron protocol needs to be re-evaluated (Svoboda & Drábek, 2005b). Piglets are now born into larger litters and are growing at a faster rate compared to previous decades, thus iron requirements may have changed from the time initial recommendations were made for supplementation. Despite all these new changes, the standard iron protocol is rarely assessed to determine if it is still sufficient in preventing anemia. Therefore, the need to reassess the iron protocol in terms of efficacy, long-term benefit, dosing and timing is needed to determine whether the current protocol is adequate in preventing iron deficiency anemia. There have been many different forms of iron supplementation practices used over the years to prevent iron deficiency anemia. The dosages of various types of iron supplementations have varied. Therefore, the re-assessment of these practices would be beneficial in determining if they are still sufficient in preventing iron deficiency anemia for modern swine practices. In the nursery, it is generally assumed that pigs will receive sufficient iron through starter diets. Typically diets contain well over the National Research Council (NRC) requirements, however there are a few studies that have evaluated iron status during this phase.
### 1.6.9 Iron Toxicity

Iron is an essential nutrient for piglets and is critical in preventing iron deficiency anemia, however excessive amounts of iron can be toxic. Sporadic reports of iron toxicity in neonatal piglets were common in the early days of iron supplementation and iron toxicity can be the result of poor quality and improper preparation of iron-dextran injections (Svoboda and Drábek, 2005b). However, the most important factor that leads to iron toxicity is vitamin E or selenium deficiency of the sow, leading to deficiency in the piglets (Svoboda and Drábek, 2005b). After injecting iron-dextran to a piglet with vitamin E deficiency, a small release of iron ions can negatively impact the piglet. Iron can lead to tissue damage by catalyzing the conversion of hydrogen peroxide to free radicals that may attack cellular membranes, proteins and DNA (Svoboda & Drábek, 2005b). The free radicals induce peroxidation of non-saturated fatty acids in cell membranes, resulting in cell membrane damage.

Piglets also may have increased potassium concentrations in blood serum due to the release of potassium from damaged muscles around the injection site caused by iron toxicity (Svoboda & Drábek, 2005b). Piglets with iron toxicity may appear anemic, weaken, are unable to stand and have muscle tremors followed by convulsions. Respiratory distress may be seen. These pigs will also have swelling around the injection site. The increase in potassium can lead to cardiac arrest and death. It has been suggested that if there is a history of iron toxicity in the herd, then an iron injection should be postponed until 24 hours after the piglet is given an injection of vitamin E (10-20 mg) (Svoboda & Drábek, 2005b). The vitamin E injection was found to prevent mortality and any adverse effects of vitamin E deficiency after an iron injection was administered.
1.7 Zinc

Zinc is considered a trace mineral in swine since pigs only require relatively low levels to promote normal growth. The recommended intake is 50-100 mg of zinc per kg of feed (NCR, 1988). The addition of 2000-3000 mg of zinc per kg of nursery feed in the form of zinc oxide is a common practice as it has been reported to improve growth rates and prevent *E. coli* diarrhea in newly weaned pigs (Rinker et al., 2005). However, the mechanism by which zinc prevents post-weaning diarrhea in newly weaned pigs is still unknown.

The increasing pressure to reduce the use of antibiotics in feed has led to the addition of high levels of zinc oxide (2000-3000 mg) in nursery rations in Ontario (Slifierz et al., 2015). Copper, iron and zinc are trace minerals that have similar physical and chemical properties. When there is an imbalance in one of these minerals it can have an antagonistic effect on the concentration of another mineral (Rinker et al., 2005). Thus, the use of high levels of zinc oxide in feed may alter the absorption of iron. Therefore, it is important to assess whether an association exits between using high levels of zinc oxide in feed and post-weaning anemia.

1.8 Hematological and Biochemical Analysis

It is often difficult and challenging to identify sick pigs due to environment conditions and the large number of pigs placed in pens. Hematological and biochemical assessment is important for many reasons in the swine industry including 1) to assess health status of a herd and production monitoring, 2) for early detection of disease, 3) diagnosing diseases, 4) for investigating hematological disorders (including anemia and
hemorrhagic diseases) and 5) to assess meat quality for human consumption (Evans, 1994). Biochemical and hematological analyses can help to identify early problems in the herd or individual pigs, thereby promptly making treatment possible. Even though hematological and biochemical assessments are very important for the above reasons, in general they are rarely used in swine practice. This is often due to the cost of laboratory work and the labour. Collecting blood can be difficult for someone who is not well trained in the procedure. The results can be inaccurate if the pig becomes stressed or excited when handled for blood collection. Also, there can be a lot of variation in hematological and biochemical values for animals considered to be of a normal health status. Within herd variation can be affected by pig age, breed and sex, as well as pathogen challenge, stress, etc. Hematological and biochemical reference ranges are important to help clinicians and researchers for interpretation of laboratory results for individual farms as well as the herd (Friendship et al., 1984). Current hematological and biochemical reference values for piglets at the time of weaning are limited.

1.9 Thesis Objectives

There are many challenges and factors that newly weaned piglets must overcome when they are brought into the nursery. These pigs are subject to multiple stressors due to new physiological, social, nutritional and environmental challenges. The overarching goal of this research was to assess and evaluate various hematological and biochemical parameters to gain a better understanding of the overall health of nursery pigs in a commercial setting.

Piglets affected with PFTS are likely anorexic for extended periods of time before being identified, and this delay in identification may influence why there are no current
diagnostic results, etiological factors or treatments known for this syndrome. It is therefore imperative to improve our ability to identify piglets in the early stages of anorexia to gain a better understanding of the risk factors associated with PFTS and anorexia in general. With this as background, the specific objectives of Chapter Two are:

- To determine if chomping pigs, defined as those piglets exhibiting excessive sham chewing, and pigs suspected to be anorexic based on a visual inspection of their body condition observed 4-7 days post-weaning have elevated serum BHB compared to non-chomping healthy pen mates;
- To compare BHB measurements taken from a handheld ketone meter on farm with standardized laboratory serum testing.

Chapter Three in the thesis evaluates iron status of piglets at weaning and then 3 weeks post-weaning. Iron supplementation occurs routinely on commercial farms, however the iron status of piglets is seldom evaluated. With updated management practices, diets and modern sow lines, sows farrow larger litters and piglets grow at an even greater rate than in previous decades (Egeli and Framstad, 1999; Jolliff and Mahan 2011). Therefore, it is important to reassess if routine iron supplementation protocols used today on commercial swine farms are still adequate in preventing iron deficiency and anemia in modern piglets. The specific objectives of this for this chapter were:

- To determine if anemia or iron deficiency is present in pigs at weaning and if it affects post-weaning performance;
- To determine if anemia or iron deficiency persists in the nursery stage;
- To determine whether high levels of zinc oxide added to starter diets are associated with the presence of anemia post-weaning.
Finally, Chapter Four aims to set benchmark values for blood parameters, both hematological and biochemistry parameters, of healthy-looking Ontario commercial pigs weaned at weaning. The specific objective of this chapter was:

- To provide updated reference ranges for hematological and biochemical parameters of Ontario commercial piglets at weaning.
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CHAPTER 2: EVALUATION OF BETA-HYDROXYBUTYRATE IN NURSERY PIGS TO HELP UNDERSTAND THE ROLE OF ANOREXIA IN PIGS AFFECTED WITH PORCINE PERIWEANING FAILURE TO THRIVE SYNDROME (PFTS)

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2.1 Abstract

Porcine periweaning failure to thrive syndrome (PFTS) is a clinical condition that affects nursery pigs, with anorexia and chomping being the main clinical signs. The objective of this study was to see if chomping pigs and pigs suspected of being anorexic observed 4-7 days post-weaning had elevated serum beta-hydroxybutyrate (BHB) compared to non-chomping pigs with good body condition. A total of 243 pigs were sampled from 4 Ontario and 4 Saskatchewan farms. Standard laboratory testing and a ketone handheld meter were used to measure BHB levels and were compared using the Bland-Altman method and non-parametric ROC analyses. Chomping and thin pigs had higher BHB values compared to non-chomping healthy pigs \( (P<0.05) \) and \( (P<0.001) \), respectively. Saskatchewan pigs had higher BHB values compared to those in Ontario \( (P<0.001) \). Both the Bland-Altman method and the ROC analysis found good agreement between diagnostic methods. The laboratory and meter BHB values were 94% correlated using a Pearson correlation.

2.2 Introduction

In commercial swine production the weaning process is a stressful transition period for piglets. At weaning piglets are moved to a new environment, switched to a solid diet and often are mixed with new pen-mates. It is well documented that within the first few days post-weaning piglets will have a significant reduction in feed consumption.
(1), growth performance, and altered intestinal function (2,3). Most piglets are able to transition well after weaning and switch onto solid feed within a few days of weaning, however, there is a subset of pigs that fail to do so. A common practice to minimize the stress of weaning is to offer creep feed to piglets in the farrowing room. This common procedure helps piglets become accustomed to solid feed while still nursing the sow. However, it is also possible that a subgroup of pigs do not consume creep feed.

Almost all pigs experience a period of reduced feed intake for a few days after weaning, however there are reports of outbreaks of debilitating anorexia in large numbers of weanling pigs on some farms (4). This clinical condition has been called periweaning failure to thrive syndrome (PFTS). Currently, there are no definitive risk factors, etiological agent(s), environmental factors or effective treatments associated with PFTS (5). A challenge with early diagnosis and treatment of PFTS-affected piglets is that they are not easily identified in the early stages of the syndrome. Pigs are likely anorexic for at least a week prior to being identified (6). The first clinical sign of PFTS is a distinct oral behaviour of excessive sham chewing (chomping) but this requires careful observation and is possibly a general sign of hunger (7). These PFTS-affected pigs and in particular the pigs that demonstrate this excessive sham chewing become lethargic and progress to severe debilitation, requiring euthanasia (4).

A previous study identified increased levels of beta-hydroxybutyrate (BHB) in anorexic nursery pigs (7). Beta-hydroxybutyrate is a serum ketone produced in the liver and is exported to peripheral tissues to use as an energy source (8). Elevated levels of ketones are formed due to fatty acid degradation occurring in the liver and this is a result
of reduced caloric intake by fasting (anorexia). The process of increased levels of ketones due to the breakdown of fat stores that are used for energy is known as ketosis.

There are few reports in the literature of ketosis in pigs, possibly because the condition is rare or because it goes undetected. However, one study evaluated ketosis in lactating sows with a handheld ketone meter because the device has been found to accurately measure BHB in dairy cattle (8). Currently there are no published studies examining ketosis in weaned piglets as a means to better understand anorexia in early post-weaning. Therefore, evaluating ketone bodies in newly weaned piglets would be beneficial in order to have a better understanding of anorexia; a common problem in the post-weaning period and a predominant clinical sign observed in PFTS-affected pigs. Also, the ability to identify anorexic pigs in a timelier manner could be important for developing interventions for anorexic pigs as well as PFTS-affected pigs.

The main objective of this study was to determine if chomping pigs, defined as those piglets exhibiting excessive sham chewing, and thin pigs suspected to be anorexic based on a visual inspection of their body condition observed 4-7 days post-weaning have elevated serum BHB compared to non-chomping pen-mates with good body condition. The second objective was to compare BHB measurements taken using a handheld meter on farm with serum testing using commercially available BHB assays performed at a veterinary diagnostic laboratory.

2.3 Materials and Methods

This project was reviewed and approved by the Animal Care Committees at the University of Guelph (permit #2429) and the University of Saskatchewan (permit
Eight commercial swine farms were conveniently selected based on willingness to participate in the study. Four farms (SASK 1, SASK 2, SASK 3, SASK 4) were located in central Saskatchewan and 4 farms (ON 5, ON 6, ON 7, ON 8) were located in southwestern Ontario. At the time of sampling, none of the 8 farms had reported observing PFTS-affected pigs in the nursery. Farms were visited to coincide with a group of pigs 4-7 days post-weaning, which corresponds to when the clinical signs of PFTS can typically be first observed and also when one is likely to see piglets experiencing anorexia or difficulty transitioning into the nursery. Thirty pigs per farm were purposefully selected based on observation of their behaviour and body condition. Two persons selected the pigs from the Saskatchewan farms and one person selected the pigs from the Ontario farms. All three individuals were experienced and highly qualified for this task. In identifying pigs for each group, all piglets in the weaned batch, regardless of batch size, were observed from outside the pen to allow the piglets to become accustomed to the presence of the observer and to minimize disruption of their behaviour. Piglets that displayed repetitive behaviour (Chomp; n=10 per farm), were identified and marked individually. These pigs were matched with a non-chomping and visually healthy pen-mate in good body condition (Control; n=10 per farm). The piglets were considered healthy if they appeared to be of good body condition, and displayed no overt clinical signs of illness such as diarrhea, coughing, or lameness. A third group of age-matched pigs were also selected that appeared thin and were suspected of being anorexic (Thin; n=10 per farm). Piglets were chosen in this manner to best simulate how a swine producer might identify sick vs. healthy piglets in a commercial setting and under practical conditions. Selected pigs were identified using a common livestock marker.
Blood sampling and beta-hydroxybutyrate (BHB) measurement

To assess if the selected piglets were ketotic whole blood samples were taken from each selected pig (n=30 per farm). Ontario pigs were bled via the orbital sinus using a Monoject™ Standard Hypodermic needle 16G x 1" (Covidien™, Mansfield, MA, USA) and collected into 8.5 mL plain tubes (BD Vacutainer®, BD, Franklin Lakes, NJ, USA). Saskatchewan pigs were bled from the jugular vein using similar consumables. Blood samples were stored in a cooler with ice packs while travelling back from each commercial farm and within a few hours after the samples were taken, the samples were centrifuged. The serum was removed and then stored at -20°C. The sera from the Ontario blood samples were shipped to Saskatchewan overnight in a cooler with dry ice and frozen cold packs. The serum samples were submitted to the Prairie Diagnostic Services, Inc., Saskatoon, Saskatchewan to measure BHB values using an Rx Monza analyzer (Randox Laboratories Ltd., Crumlin, County Atrium, UK). This method involves the oxidation of beta-hydroxybutyrate to acetoacetate via the enzyme 3-hydroxybutyrate dehydrogenase. The overall change in absorbance is from the reduction of NAD⁺ to NADH. This change in absorbance is directly correlated with BHB concentration.

BHB levels were also measured in real-time using an Abbott Precision Xtra blood (Abbott Diabetes Care Inc., Alameda, CA, US) handheld ketone meter while on the farm. This handheld device is an easy and direct electrochemical test. This device provides a BHB measurement (mmol/L) within 10 sec after 1.5 uL of blood has been applied to the Precision Xtra blood ketone test strip. The test strip contains the enzyme beta-hydroxybutyrate dehydrogenase, which oxidizes BHBA to acetoacetate. This process
reduces \( \text{NAD}^{+} \) to \( \text{NADH} \). The \( \text{NADH} \) is then re-oxidized by an electron mediator molecule and the electrical current that is created by the conversion is measured by the meter and directly proportional to the BHB concentration (9).

**Statistical Analysis**

*Association between beta-hydroxybutyrate and anorexia:*

Various statistical models and methods were used in STATA 12.0 (Stata 12 Statacorp LP, College Station, Texas, USA) and SPSS Version 22 (SPSS Inc., Chicago, IL, USA) to examine associations between ketosis, body condition and the oral behaviour of chomping.

A linear regression was initially designed to examine the association between BHB levels as a continuous variable, while controlling for pig assignment (Control, Chomp, Thin), farm, and province as fixed effects. A mixed linear regression was also created to account for clustering at the farm level as a random effect while controlling for pig assignment (Control, Chomp, and Thin), and province as a fixed effect. However, the assumptions of normality and homoscedasticity were not met for either model. Homoscedasticity was assessed graphically and statistically using the Cook-Weisberg test in STATA12.0. Normality was assessed graphically and statistically using the Shapiro-Wilk’s test. Various transformations were also assessed (square root, log, and reciprocal), however they did not improve model fit.

Subsequently, a logistic regression was designed to evaluate the association between ketotic pigs as a dichotomous variable, while controlling for pig category (Control, Chomp, and Thin), farm and province as fixed effects. Pigs with BHB values >
0.1 mmol/L were defined as ketotic and pigs with BHB values ≤0.1 mmol/L were defined as normal. However, when evaluating the fit of the model using the Pearson goodness of fit test in STATA 12.0, the model did not fit the data. Due to the small sample size a more robust exact logistic regression model was also created, however due to the lack of computational power farms could not be modeled as a fixed effect. Therefore, this model was not considered further.

Due to the lack of fit in the models described above, non-parametric tests were then used to analyze the data since they do not require the assumption of a Gaussian distribution. All non-parametric tests were developed using SPSS Version 22. In all non-parametric models created, the laboratory results were used as the gold standard. The Kruskal-Wallis test was used to assess whether the mean ranks of BHB values from both the laboratory and ketone meter were different among pig groups (Control, Chomp, and Thin). The Kruskal-Wallis test was also used to assess the mean ranks of BHB values from all participating farms. The Mann-Whitney U tests were used to compare the mean ranks of BHB values between provinces, as well as three group contrasts: 1) Chomp versus Thin, 2) Chomp versus Control and 3) Thin versus Control.

*Evaluation of diagnostic test agreement*

Test agreement between the Precision Xtra and the Rx Monza BHB values was evaluated using a Bland-Altman plot using STATA 12.0. This graphical method plots the differences between both methods against the average of both methods on a continuous scale. This method calculates the 95% confidence intervals and the mean difference. It is expected the limits of agreement contain 95% of the differences between the handheld
meter and the laboratory BHB values. A Pearson correlation coefficient was then evaluated to assess the strength of association of laboratory and meter BHB values.

Additionally, three non-parametric receiver operating characteristic (ROC) curves were used to assess the overall ability to discriminate ketotic pigs from non-ketotic pigs based on BHB values measured using both techniques. A non-parametric ROC curve was used because the data set did not follow a Gaussian distribution. A ROC curve is used to illustrate the characteristics of a diagnostic test by graphing the false-positive rate (1-specificity) on the x-axis and the true-positive rate (sensitivity) on the y-axis for different cut-off values. The ROC curves were developed in STATA 12.0. Pigs were defined as ketotic if their Rx Monza BHB values were ≥0.1 mmol/L, and were used as the reference variable (gold standard) for the non-parametric ROC analysis. Each pig was identified as either ketotic or normal as a dichotomous variable (0=normal, 1=ketotic). When using the Precision Xtra to define pigs as ketotic, three different cut-points were assessed: 1) using the same cut-point as the Rx Monza (ketotic if BHB values were ≥0.1 mmol/L), 2) classifying pigs as ketotic if BHB values were ≥0.2 mmol/L and 3) classifying pigs as ketotic if BHB values were ≥0.3 mmol/L. Pigs were categorized as 0 (normal BHB values), 1 (BHB values ≥0.1 mmol/L), 2 (BHB values ≥0.2 mmol/L), and 3 (BHB values ≥0.3 mmol/L). In the first ROC curve, the Precision Xtra and Rx Monza BHB measurements were compared using a cut-point of BHB values ≥0.1 mmol/L to define ketosis. In the second ROC curve, a cut-off of ≥0.2 mmol/L was used for the Precision Xtra when compared against the Rx Monza results using ≥0.1 mmol/L as the cut-off. The third ROC curve compared a cut-off of ≥0.3 mmol/L by the Precision Xtra when compared against the Rx Monza results using ≥0.1 mmol/L as the cut-off. The accuracy
of the Precision Xtra depends on its ability to measure BHB. The accuracy is measured by calculating the area under the ROC curve (AUC). The points on the ROC curve are then connected using straight lines and the AUC is computed using the trapezoidal rule. An estimate of the variance for the AUC is calculated using the DeLong method (10). Arbitrary cut-offs are then used to classify pigs as ketotic based on BHB levels.

2.4 Results

A total of 243 pigs were sampled (30 pigs each from 7 farms, 33 pigs from SASK 2). Of the 243 pigs sampled, 3 pigs from one farm (SASK 2) had missing Precision Xtra BHB measurements, because insufficient test strips were available. Nursery pigs selected varied in breed and all male pigs enrolled in this study had been castrated. The prevalence of ketosis by pig group (Chomp, Thin, Control) based on Precision Xtra and Rx Monza BHB measurements is illustrated in Table 2.1. The frequency of pigs defined as having normal BHB values compared to ketotic pigs using the Rx Monza values is shown in Figure 2.1. The frequency of pigs with normal and ketotic BHB values using the Precision Xtra between pig groups is presented in Figure 2.2. A difference was found in the BHB values measured by the Rx Monza between the three pig groups ($P<.001$). The mean ranks for Chomp, Thin and Control pigs are 126.5, 138.2 and 100.7, respectively (Table 2.2). Post-hoc contrasts indicated Chomp pigs had higher BHB values compared to Control pigs ($P<0.05$) and Thin pigs had higher BHB values compared to the Control pigs ($P<0.001$). When comparing the BHB values between Chomp and Thin pigs, there was no significant difference found (Table 2.3).
When assessing the mean ranks among all participating farms and BHB values, a difference in BHB values was found between farms ($P < 0.001$). The assessment of BHB values between provinces using both the Rx Monza and Precision Xtra are presented in Table 2.4. The frequency of pigs defined as ketotic by pig group based on Rx Monza and Precision Xtra BHB values are illustrated in Figures 2.1 - 2.2.2. The number of normal and ketotic pigs by province based on Rx Monza BHB levels and Precision Xtra BHB levels are presented in Figures 2.3 – 2.4.2. Nursery pigs in Saskatchewan had higher BHB values compared to nursery pigs in Ontario ($P < 0.001$). Thus, there were more pigs from Saskatchewan that were ketotic compared to Ontario pigs and this is presented in Table 2.5.

The Bland-Altman plot presented in Figure 2.5, evaluates the agreement between Rx Monza and Precision Xtra BHB values. The mean and standard deviation (±SD) of the Rx Monza and Precision Xtra BHB values are $0.14 ± 0.16$ mmol/L and $0.17 ± 0.22$ mmol/L, respectively. The grey box represents the 95% confidence limits (limits of agreement). The mean difference between the Precision Xtra and Rx Monza BHB values is $-0.034$. This value is known as the bias. The SD for the differences between the BHB values for both methods is 0.086. The upper limit of agreement was computed as $1.96 (2$ SD) times $0.086$ (SD) plus the bias between both methods ($-0.034$). The upper limit of agreement is $0.134$ mmol/L. The lower limit of agreement is $1.96 (2$ SD) times $0.086$ (SD) minus the bias between both methods ($-0.034$). The lower limit of agreement is $-0.202$ mmol/L. Therefore, if the differences between the Precision Xtra and the Rx Monza BHB values are distributed normally, 95% of the differences are expected to be between $-0.202$ and $0.134$ mmol/L. The confidence limit ($0.134$ to $-0.202$ mmol/L) is
0.336 mmol/L. It is noted that 8 of 240 (3.3%) data points exceed the lower limits of agreement. These 8 data points were all from pigs in the same farm (Farm 4) in Saskatchewan. Since less than 5% of pigs exceeded the upper and lower limits of agreement, this test suggests that the Precision Xtra and the Rx Monza results had relatively good agreement. The Pearson correlation between the Rx Monza and Precision Xtra BHB values was $r=0.94$. This indicates that the results from both techniques were highly correlated.

The first non-parametric ROC curve generated compared both the Precision Xtra and Rx Monza BHB values to assess a cut-off point of $\geq 0.1$ mmol/L to categorize ketotic pigs and is illustrated in Figure 2.6. Based on the Rx Monza, 16 of 243 pigs were classified as ketotic (BHB values $\geq 0.1$ mmol/L), while as the Precision Xtra classified 87 of the 240 pigs as ketotic. When using the Precision Xtra and a cut-off BHB $\geq 0.1$ mmol/L for defining ketosis, the sensitivity and specificity were 100% and 68.4% respectively. Using this cut-off point, 70.5% of the 240 pigs were correctly classified. The AUC is 0.842 and the standard error is 0.015, (CI: 0.812-0.873).

The second ROC curve assessed a Precision Xtra cut-off of $\geq 0.2$ mmol/L with the $\geq 0.1$ mmol/L Rx Monza BHB cut-off and is presented in Figure 2.6.1. When using this second Precision Xtra cut-off point for categorizing ketotic pigs, 24 of the 240 pigs were defined as ketotic. When using this cut-off point for categorizing ketotic pigs, the sensitivity and specificity were 100.0% and 96.4% respectively. This cut-off point, classified 96.7% of the 240 pigs correctly. The AUC is 0.982 and the standard error is 0.006, (CI: 0.970-0.994).
The third ROC curve assessed a Precision Xtra cut-off of $\geq 0.3$ mmol/L with the $\geq 0.1$ mmol/L Rx Monza BHB cut-off and is presented in Figure 2.6.2. This cut-off point categorized 16 of the 240 pigs as ketotic. When assessing this last cut-off point, the sensitivity and specificity were 81.3% and 98.7% respectively. Of the 240 pigs sampled, 97.5% were correctly classified. The AUC is 0.900 and the standard error is 0.051, (CI: 0.801-0.999). Based on the analysis above, the most effective cut-off point for determining ketosis on farm when using the Precision Xtra is using BHB values $\geq 0.2$ mmol. This cut-off value had the greatest accuracy, as determined by the AUC. Also, this cut-off value classified all ketotic pigs since the sensitivity was 100% with limited false positives. Therefore, having 100% sensitivity as well as a high specificity will allow for pigs to be quickly identified and treated accordingly.

2.5 Discussion

This study found that chomping pigs, defined as those pigs exhibiting excessive sham chewing, and thin pigs suspected of being anorexic based on a visual inspection of their body condition observed 4-7 days post-weaning had elevated serum BHB compared to non-chomping pen mates that are in good body condition. The ability to identify a significant difference in serum BHB between the 3 piglet groups is important for developing a benchmark for normal BHB levels as well as elevated levels in newly weaned pigs. A province effect was also found in this study when comparing serum BHB values between Ontario and Saskatchewan farms. There was however one farm in Saskatchewan that was included in the study that was affected with swine influenza, which significantly affected the health status of the nursery piglets at the time of
sampling. In fact, this farm had higher mean rank BHB values for both the Chomp and Thin pigs compared to the other seven farms, which indicates that these pigs were not in good health, which could have affected their BHB levels. However, the remaining 3 Saskatchewan farms also had higher BHB values compared to the Ontario farms. This could indicate that the Saskatchewan farms selected may have had more weaning challenges for the pigs to overcome or were also experiencing other disease challenges compared to the Ontario farms. Since there are no previous studies published in the literature that have assessed serum BHB in weanling pigs, the findings of this study provides a baseline and supports that serum BHB might prove to be useful in monitoring herd outbreaks of various swine diseases. For instance, in this current study pigs from the affected farm with swine influenza had higher serum BHB values compared to all other farms. Thus, evaluating serum BHB can be important for the early identification of other diseases such as PFTS and porcine reproductive and respiratory syndrome (PRRS), since piglets may initially reduce or stop consuming feed prior to characteristic clinical signs such as coughing, nasal discharge or loss of notable body weight. Therefore, rather than waiting for clinical signs of a disease to occur, evaluating serum BHB in weaned pigs could be useful for indicating something that may be going on with a herd and which can prompt further action.

This study found that BHB measurements taken with the Abbott Precision Xtra blood ketone handheld meter on farm showed moderate to good agreement with standardized laboratory serum testing. The value of testing on farm is that it is convenient to use, more cost effective, and access to the analytical results is immediate compared to submitting samples to a laboratory and waiting for results. A limitation to this study is
that there are no previous studies that have evaluated serum BHB in nursery pigs and therefore it is not possible to compare these findings with other studies. However, the Abbott Precision Xtra blood ketone handheld meter has been used on cattle and found to have relatively accurate results. In three previous studies, the results were all very similar and comparable to each other (11,12,13). In total, the 3 studies looked at 622 cows with a 14.1% prevalence of ketosis (9). The average coefficient of determination ($R^2$) when comparing the ketone handheld meter and the laboratory BHB results was 0.94. The sensitivity of the meter was 91% and the specificity was 94% for diagnosing ketosis (pooled results between all three trials) (9). The positive and negative predictive values for the ketone handheld meter when using pooled results from the 3 trials was 73% and 98%, respectively (9). The handheld meter was used in this study to evaluate a pen-side diagnostic test and to assess its on-farm utility and accuracy. There are many advantages to using the ketone handheld meter for herd-based monitoring. The cost of ketone test strips are less than the cost of laboratory testing, the result is known immediately, only a small drop of blood is required and there is no need to ship or process serum or plasma samples to laboratories.

The current study investigates whether measuring serum BHB would help identify anorexic pigs within the first 4-7 days post-weaning possibly quicker than by using clinical signs such as chomping or loss of body condition (clinical signs of PFTS). Ketone levels were assessed to see whether a difference could be found between pigs that were thin and possibly anorexic (Thin), pigs that exhibited a repetitive oral behaviour of sham chewing (Chomp) and pigs that show no obvious signs of disease, have a good body condition and do not display chomping behaviour (Control). These pigs that fail to
transition to the nursery phase are typically healthy during the suckling phase and develop partial to complete anorexia and subsequently decline in body condition. However, within the first week of weaning the decline in body condition from the anorexic pigs or those exhibiting a reduction in feed intake is often subtle and it is likely that producers cannot identify anorexic pigs at this time. An early indication that a pig is not eating is chomping but that requires a very observant worker and can be easily missed. A complexity associated with identifying PFTS-affected pigs is that these pigs are likely anorexic for prolonged periods of time without being visually identified in the early stages of the syndrome (6). This delay in identifying anorexia in PFTS-affected pigs has likely played a role in the inconclusive diagnostics and lack of response to treatments reported on farms to date. Thus, it is important to assess serum BHB to enable more rapid diagnosis of anorexia and a more thorough understanding of PFTS.

As expected a priori, by the time pigs were thin because of reduced feed intake or anorexia, the serum BHB test was able to identify this group of pigs with the highest BHB mean ranks. Pigs thought to be anorexic based on the clinical sign of chomping also showed elevated serum BHB. However, it is important to note that of the pigs selected 94% and 87% of the Chomp and Thin pigs, respectively had normal Rx Monza BHB levels. It is also likely that the majority of pigs were not completely anorexic, as they may be eating some food, but their average daily feed intake (ADFI) is decreased. Therefore, not all Chomp and Thin pigs were ketotic, suggesting these pigs were not completely anorexic. This indicates that experienced technicians are not able to accurately select pigs that were ketotic based on behavioural and body condition observations. Thus, these clinical signs may not be very helpful for identifying ketotic pigs. Since chomping is a
predominate clinical sign of PFTS, being able to identify pigs that are ketotic and suspected to be anorexic in the early stage of PFTS will further help to impose diagnostic tests in the earlier stages of the syndrome. Thus, if a producer was unable to correctly identify thin pigs or to patiently observe chomping pigs, a blood test could be used to identify anorexic pigs in the early stages of the problem.

When comparing the BHB values between the Rx Monza and Precision Xtra using the Bland-Altman method, visually the test agreement is moderate to good. However, this is a preliminary study with no a priori criteria to compare the results to. Also, since this is the first study to assess ketones in nursery pigs, there are no reference values for either methodology. The mean and SD of the values obtained with the Precision Xtra were slightly higher compared to the values obtained using the Rx Monza, indicating that the former overestimates the BHB values. However, the Pearson correlation coefficient was very high when comparing the methodologies, therefore the handheld meter has utility for sampling on farm.

Similar findings were found when evaluating the BHB values between the Rx Monza and Precision Xtra using the ROC curve for analysis. When comparing the Precision Xtra BHB values and the Rx Monza values using the ≥0.1 mmol/L cut-point for defining ketosis, approximately 70.5% of the Precision Xtra BHB values agreed with the Rx Monza BHB values which indicates moderate agreement. When the meter cut-point was increased to ≥0.2 mmol/L the AUC increased to approximately 97% indicating almost perfect agreement. The results from this study indicate that using a BHB cut-off value of ≥0.2 mmol/L when using the Precision Xtra can produce comparable results to Rx Monza BHB testing with a cut-off value of ≥0.1 mmol/L. As mentioned earlier, there
is no literature assessing ketosis in nursery pigs and therefore no standard diagnostic measurements for reference ranges to evaluate ketosis. Therefore, there is sufficient data to enable the use of the Precision Xtra on farm, while using a cut-off value between 0.1-0.3 mmol/L depending on the sensitivity and specificity needed. This will be dependent on the goals of testing on a case-by-case basis for veterinarians.

There are a few limitations to this study. Firstly, when selecting pigs from both Ontario and Saskatchewan farms, multiple individuals selected the pigs. Although the individuals are experienced in identifying chomping behaviour and thin pigs, there could be some selection bias in this study design due to not having the same individual selecting all the pigs. When selecting the pigs, the pigs were observed for 5 minutes per pen. This may not be long enough when searching for piglets that display repetitive chomping. Also, only a small number of pigs in the study were ketotic. The sample size of this study was small and this may be a reason the mean rank Rx Monza BHB values did not differ in the Thin and Chomp groups.

In summary, this study identified serum BHB differences between newly weaned pigs that were healthy and in good body condition, pigs exhibiting chomping behaviour, and thin pigs. The latter two groups were selected because loss of body condition and chomping are the two most commonly used clinical signs to indicate pigs that are possibly anorexic. The thin pigs as well as the chomping pigs had elevated BHB values compared to their matched healthy pen-mates. However, many of the pigs chosen based on clinical signs to be anorexic were found to have BHB values similar to the control pigs with healthy body conditions. Our conclusion from this study is that clinical signs of chomping and thinness do not necessarily identify pigs that are anorexic. Some of these
pigs had elevated BHB, but most were normal. Thus, BHB measurement is the more accurate measure of anorexia compared to identifying clinical signs. The pen-side handheld ketone meter, Abbot Precision Xtra, had moderate to good agreement compared to BHB values obtained from the same pigs using standardized laboratory serum testing methodology, the Rx Monza assay. Thus, the handheld ketone meter is a practical method to identify anorexic pigs quickly on farm and this may lead to a better understanding of anorexia in newly weaned piglets associated with PFTS and other swine diseases. Due to the lack of research publications on ketosis in swine, there is no known reference range for BHB values in weanling pigs. Therefore, future work is needed to develop reference ranges to enable a more accurate diagnosis of ketosis in nursery pigs.
2.6 References


Table 2.1: Number and percentage of ketosis by pig group (Control, Chomp, Thin) using different beta-hydroxybutyrate (BHB) cut-off values

<table>
<thead>
<tr>
<th>Cut-off Points for BHB measurements</th>
<th>Sample Size</th>
<th>Normal BHB levels</th>
<th>Ketotic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rx Monza ≥0.1 mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Pigs</td>
<td>80</td>
<td>100.00%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=80)</td>
<td>(n=0)</td>
</tr>
<tr>
<td>Chomp Pigs</td>
<td>80</td>
<td>93.75%</td>
<td>6.25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=75)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Thin Pigs</td>
<td>83</td>
<td>86.75%</td>
<td>13.25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=72)</td>
<td>(n=11)</td>
</tr>
<tr>
<td><strong>Precision Xtra ≥0.1 mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Pigs</td>
<td>80</td>
<td>71.25%</td>
<td>28.75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=57)</td>
<td>(n=23)</td>
</tr>
<tr>
<td>Chomp Pigs</td>
<td>80</td>
<td>68.75%</td>
<td>31.25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=55)</td>
<td>(n=25)</td>
</tr>
<tr>
<td>Thin Pigs</td>
<td>83</td>
<td>53.01%</td>
<td>46.99%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=44)</td>
<td>(n=39)</td>
</tr>
<tr>
<td><strong>Precision Xtra ≥0.2 mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Pigs</td>
<td>80</td>
<td>95.00%</td>
<td>5.00%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=76)</td>
<td>(n=4)</td>
</tr>
<tr>
<td>Chomp Pigs</td>
<td>80</td>
<td>91.25%</td>
<td>8.75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=73)</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Thin Pigs</td>
<td>83</td>
<td>84.34%</td>
<td>15.66%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=70)</td>
<td>(n=13)</td>
</tr>
<tr>
<td><strong>Precision Xtra ≥0.3 mmol/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Pigs</td>
<td>80</td>
<td>96.25%</td>
<td>3.75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=77)</td>
<td>(n=3)</td>
</tr>
<tr>
<td>Chomp Pigs</td>
<td>80</td>
<td>93.75%</td>
<td>6.25%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=75)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Thin Pigs</td>
<td>83</td>
<td>90.36%</td>
<td>9.64%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n=75)</td>
<td>(n=8)</td>
</tr>
</tbody>
</table>
Table 2.2: Mean ranks of beta-hydroxybutyrate (BHB) values by pig group for both the Rx Monza and Precision Xtra diagnostic tools

<table>
<thead>
<tr>
<th>Method</th>
<th>Pig Group</th>
<th>Sample size</th>
<th>Mean Rank(a)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rx Monza</td>
<td>Control</td>
<td>80</td>
<td>100.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Chomp</td>
<td>80</td>
<td>126.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thin</td>
<td>83</td>
<td>138.2</td>
<td></td>
</tr>
<tr>
<td>Precision Xtra</td>
<td>Control</td>
<td>77</td>
<td>107.4</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Chomp</td>
<td>80</td>
<td>118.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thin</td>
<td>83</td>
<td>134.8</td>
<td></td>
</tr>
</tbody>
</table>

\(a\)Mean rank obtained using Kruskal Wallis Test (SPSS v22)
Table 2.3: Pairwise contrasts of mean ranks of beta-hydroxybutyrate (BHB) Rx Monza values

<table>
<thead>
<tr>
<th>Pairwise Contrast</th>
<th>Sample Size</th>
<th>Mean Rank$^a$</th>
<th>Sum of Ranks</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chomp v. Thin</td>
<td>80</td>
<td>78.0</td>
<td>6241.5</td>
<td>0.275</td>
</tr>
<tr>
<td>Chomp v. Control</td>
<td>80</td>
<td>89.0</td>
<td>7120.0</td>
<td>0.014</td>
</tr>
<tr>
<td>Thin v. Control</td>
<td>83</td>
<td>94.4</td>
<td>7832.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^a$Mean rank obtained using Mann-Whitney U test (SPSS v22)
Table 2.4: Mean ranks of beta-hydroxybutyrate (BHB) values by province obtained using the Rx Monza and Precision Xtra diagnostic tools

<table>
<thead>
<tr>
<th>Method</th>
<th>Province</th>
<th>Sample Size</th>
<th>Mean Rank $^a$</th>
<th>Sum of Ranks</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rx Monza</td>
<td>Saskatchewan</td>
<td>123</td>
<td>145.8</td>
<td>17929.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Ontario</td>
<td>120</td>
<td>97.6</td>
<td>11716.5</td>
<td></td>
</tr>
<tr>
<td>Precision Xtra</td>
<td>Saskatchewan</td>
<td>120</td>
<td>145.9</td>
<td>17512.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Ontario</td>
<td>120</td>
<td>95.1</td>
<td>11407.5</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Mean rank obtained using a Mann-Whitney U test (SPSS v22)
Table 2.5: Mean ranks of beta-hydroxybutyrate (BHB) values by farms and province for both the Rx Monza and Precision Xtra diagnostic tools

<table>
<thead>
<tr>
<th>Farm</th>
<th>Sample Size</th>
<th>Rx Monza Mean Rank&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Precision Xtra Mean Rank&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SASK 1</td>
<td>n=30</td>
<td>132.9</td>
<td>&lt;0.001</td>
<td>127.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SASK 2</td>
<td>n=33</td>
<td>130.0</td>
<td></td>
<td>138.3</td>
<td></td>
</tr>
<tr>
<td>SASK 3</td>
<td>n=30</td>
<td>138.0</td>
<td></td>
<td>132.2</td>
<td></td>
</tr>
<tr>
<td>SASK 4</td>
<td>n=30</td>
<td>183.8</td>
<td></td>
<td>184.7</td>
<td></td>
</tr>
<tr>
<td>ON 5</td>
<td>n=30</td>
<td>75.0</td>
<td></td>
<td>83.7</td>
<td></td>
</tr>
<tr>
<td>ON 6</td>
<td>n=30</td>
<td>103.1</td>
<td></td>
<td>101.9</td>
<td></td>
</tr>
<tr>
<td>ON 7</td>
<td>n=30</td>
<td>103.8</td>
<td></td>
<td>88.3</td>
<td></td>
</tr>
<tr>
<td>ON 8</td>
<td>n=30</td>
<td>108.7</td>
<td></td>
<td>106.4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean rank obtained using a Kruskal Wallis Test (SPSS v22)

<sup>b</sup> The Rx Monza BHB Mean Ranks are significantly different between the 8 participating farms (P<0.001)

<sup>c</sup> Mean rank obtained using a Kruskal Wallis Test (SPSS v22)

<sup>d</sup> The Precision Xtra BHB Mean Ranks are significantly different between the 8 participating farms (P<0.001)
Figure 2.1: Number of normal (non-ketotic) and ketotic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on Rx Monza beta-hydroxybutyrate (BHB) measurements.

*Ketosis defined as BHB blood values $\geq 0.1$ mmol/L.
*Error bars for 95% confidence intervals.
Figure 2.2: Number of normal (non-ketotic) and ketotic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on the Precision Xtra beta-hydroxybutyrate (BHB) values ≥0.1 mmol/L cut-off.

^Ketosis defined as BHB blood values ≥0.1 mmol/L.

^Error bars for 95% confidence intervals
Figure 2.2.1: Number of normal (non-ketotic) and ketotic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on the Precision Xtra beta-hydroxybutyrate (BHB) values ≥0.2 mmol/L cut-off.

*Ketosis defined as BHB blood values ≥0.2 mmol/L

Error bars for 95% confidence intervals
Figure 2.2.2: Number of normal (non-ketotic) and ketogenic pigs by pig group from 8 commercial farms in Ontario and Saskatchewan based on the Precision Xtra beta-hydroxybutyrate (BHB) values ≥0.3 mmol/L cut-off.

Ketotic pigs have blood BHB values ≥0.3 mmol/L.

Error bars for 95% confidence intervals.
Figure 2.3: Number of normal (non-ketotic) and ketotic pigs by province based on Rx Monza beta-hydroxybutyrate (BHB) levels

*Ketotic pigs have blood BHB values ≥0.1 mmol/L
*Error bars for 95% confidence intervals
Figure 2.4: Number of normal (non-ketotic) and ketotic pigs by province based on Precision Xtra beta-hydroxybutyrate (BHB) levels ≥0.1 mmol/L cut-off

*Ketotic pigs have blood BHB values ≥0.1 mmol/L

*Error bars for 95% confidence intervals
Figure 2.4.1: Number of normal (non-ketotic) and ketotic pigs by province based on Precision Xtra beta-hydroxybutyrate (BHB) levels ≥0.2 mmol/L cut-off

Ketotic pigs have BHB blood values ≥0.2 mmol/L

Error bars for 95% confidence intervals
Figure 2.4.2: Number of normal (non-ketotic) and ketotic pigs by province based on Precision Xtra beta-hydroxybutyrate (BHB) levels ≥0.3 mmol/L cut-off

Ketotic pigs have blood BHB values ≥0.3 mmol/L

Error bars for 95% confidence intervals
Figure 2.5: Bland-Altman plot evaluating agreement between Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values using ≥0.1 mmol/L cut-off value for defining ketosis.

8/240 = 3.33% outside the limits of agreement
bMean difference = -0.034
95% limits of agreement (-0.202, 0.134)
Averages lies between 0.050 and 1.675 mmol/L
Outliers labeled by farm number
All outliers from farm SASK 4
Figure 2.6: A non-parametric ROC curve assessing the agreement between the Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values with a ≥0.1 mmol/L cut-off value for defining ketosis.

.area under ROC curve = 0.842
Figure 2.6.1: A non-parametric ROC curve\textsuperscript{a} assessing the agreement between the Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values with a $\geq 0.2$ mmol/L cut-off value for defining ketosis.

\textsuperscript{a}Area under ROC curve = 0.982
Figure 2.6.2: A non-parametric ROC curve assessing the agreement between the Rx Monza and Precision Xtra beta-hydroxybutyrate (BHB) values with a ≥0.3 mmol/L cut-off value for defining ketosis

Area under ROC curve = 0.899

aArea under ROC curve = 0.899
CHAPTER 3: AN INVESTIGATION OF IRON DEFICIENCY AND ANEMIA IN PIGLETS AND THE EFFECT OF IRON STATUS AT WEANING ON POST-WEANING PERFORMANCE

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3.1 Abstract

Objectives: To determine if anemia or iron deficiency is present in pigs at weaning and if it affects post-weaning performance. Also, to determine if anemia or iron deficiency persists in the nursery stage and if so whether high levels of zinc oxide in-feed is associated with post-weaning anemia.

Materials and methods: The study involved 20 Ontario swine farms and 1095 pigs were sampled. A small, medium, and large piglet were selected per litter and serum, whole blood samples, and body weights were taken at weaning. Three weeks later the same pigs were re-weighed and whole blood samples taken. Hemoglobin and a complete blood count (CBC) were analyzed to assess iron status and their associations with post-weaning performance. A questionnaire regarding iron supplementation protocols and level of zinc in nursery feed was administered to each producer.

Results: Despite routine parenteral iron supplementation, anemic and iron deficient pigs could be found at weaning on almost all participating farms. Anemic pigs at weaning were 0.82 kg lighter at 3-week post-weaning compared to piglets with normal hemoglobin values at weaning (P <.05). Larger piglets at weaning had lower hemoglobin, serum iron, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration levels and higher total iron binding capacity values when compared to smaller piglets (all P<.05). There was a higher prevalence of
anemic pigs 3 weeks post-weaning than at weaning, and prevalence of anemia was associated with feeding high levels of zinc oxide (>2000 mg/kg).

**Implications:** Current iron supplementation protocols used by the study herds were inadequate to prevent iron deficiency, particularly in the largest pigs. Anemic pigs at weaning have slower growth rates when compared to pigs with adequate iron status at weaning. The consumption of starter feeds in the nursery does not prevent pigs from becoming anemic after weaning, particularly if the feed contains high levels of zinc oxide.

### 3.2 Introduction

It is well established that insufficient intake of iron in suckling pigs results in iron deficiency or anemia, where the concentration of hemoglobin (Hb) and the number and size of red blood cells (RBCs) decline below the normal range.\(^1\) The suckling pig, regardless of breed, is susceptible to iron deficiency, anemia or both\(^2,3\) because: 1) the pig is born with limited iron, approximately 50 mg, mostly incorporated in hemoglobin\(^4\) 2) sow milk is a poor source of iron, providing piglets with only 1 mg of iron a day,\(^4\) 3) pigs lack access to soil due to confinement rearing indoors, and 4) the modern pig has been selected for rapid growth.\(^4\) In the first week of life, piglets double their weight and increase their plasma volume by 30%, thereby diluting the concentration of Hb.\(^5\) The daily iron requirement of iron for piglets is approximately 7 mg and therefore, the limited iron that piglets are born with are inadequate in preventing iron deficiency and anemia since these body stores deplete very rapidly.\(^4,6-9\)
Hemoglobin (Hb) is commonly used as a measurement of iron status because 80-90% of the iron present in the suckling piglet is used in forming Hb. Hemoglobin is an important protein involved in cellular metabolism, as it transports oxygen from the lungs to other body tissues and carbon dioxide back to the lungs for expulsion via the respiratory tract. Iron deficient and anemic pigs have fewer RBCs containing less Hb compared to piglets with normal Hb levels. Along with Hb, other iron indicators such as serum iron levels and total iron binding capacity (TIBC) are also important for assessing iron status in swine. Serum iron measures the amount of iron circulating in the blood bound to transferrin. Transferrin is an important protein that binds and transports iron in blood. Total iron binding capacity measures the blood’s capacity to bind with transferrin.

Piglets require exogenous iron supplementation within the first week of life to compensate for their limited iron and to prevent iron deficiency and/or anemia. It is commonly recommended to administer a 200 mg intramuscular (IM) injection of iron dextran within the first 3 days of life. Although oral iron is sometimes used, parenteral administration of iron is the most common method of iron supplementation for pigs on commercial swine farms. Iron supplementation is carried out on a routine basis on commercial farms however the iron status of piglets is seldom evaluated. With updated management practices and modern sow lines, sows farrow larger litters and piglets grow at an even greater rate than in previous decades. Therefore, it is imperative to reassess if routine iron supplementation protocols used today on commercial swine farms are still adequate in preventing iron deficiency and anemia in modern piglets. This could have animal health and economic implications as piglets that have inadequate iron stores can develop a suppressed immune system resulting in an impaired ability to resist
infectious and parasitic diseases, leading to slower growth rates and increased morbidity and mortality.\textsuperscript{12}

Iron deficiency occurs when there is a reduction in the total content of iron in the body.\textsuperscript{15} When there is a lack of iron in the body, nutrient requirements are not met. During the early stages, clinical signs such as anemia may not be apparent. Whereas, anemia occurs when iron deficiency is severe and leads to a reduction in erythropoiesis.\textsuperscript{15} The reason for the occurrence of iron deficiency, anemia or both on commercial swine farms may be due to husbandry errors i.e. inadequate dosing or timing of administration of iron, or it may be that modern piglets require a higher dosage of iron during routine iron supplementation procedures. Also, modern swine practices such as the use of high levels (>2000 mg/kg) of zinc oxide in the feed to control \textit{Escherichia coli} diarrhea in newly weaned pigs, may decrease iron absorption. Copper, iron and zinc are trace minerals that have similar physical and chemical properties. When there is an imbalance in one of these minerals it can have an antagonistic effect on the nutritional availability of another mineral.\textsuperscript{16} Thus, the use of elevated levels of zinc oxide (≥ 500 mg/kg) in feed may alter the absorption of iron.

The economic impact of inadequate iron supplementation in piglets is unknown, however, iron status can be easily evaluated and corrected for minimal additional cost. It costs approximately 15 CAD\$/pig for standard laboratory testing and 1 CAD\$/pig using a handheld hemoglobin meter. Moreover, any impact iron deficiency may have on growth rates could negatively affect the cost of production. Therefore, the minimal added cost associated with an evaluation and correction of iron deficiency or anemia could likely outweigh the benefits of improved weight gain and improved welfare of the piglets.
The objectives of this study are to determine if anemia or iron deficiency is present in pigs at weaning and if it affects post-weaning performance, and to determine if anemia or iron deficiency persists in the nursery stage and if so whether high levels of zinc oxide added to starter feeds is associated with the presence of post-weaning anemia.

### 3.3 Materials and Methods

**Study Design & Sampling:**

The Animal Care Committee at the University of Guelph, that follows the guidelines of the Canadian Council for Animal Care, reviewed and approved this study (permit #2429). Twenty swine farms from 10 different counties across southern Ontario were enrolled. The farms were conveniently sampled to represent a wide variety of production types, management practices, and sow-herd size. A questionnaire (presented in Appendix I) was administered at each farm to collect information regarding iron supplementation practices including the age of piglet at the time of administration, dose and type of iron supplementation product(s) used. The questionnaire also captured farm management information such as the size of the sow herd, weaning age and pig flow.

Each farm was visited twice. At the first visit, which occurred 1 to 2 days prior to weaning, litters were systematically selected starting at the first crate in the farrowing room until a maximum of 20 litters per farm was reached. Three piglets per litter were purposefully selected based on their size and included one large, one medium and one small piglet per litter based on visual assessment. Pigs were excluded if they exhibited any physical abnormalities such as an abscess or hernia, or if they were unthrifty e.g. thin body condition, or lame. Piglets were selected in this manner to obtain a balanced sample
of different sized piglets to enable the assessment of iron status by body size. Each
selected piglet was individually ear tagged and weighed (DYMO® Shipping Scale S100,
Atlanta, GA, USA). Blood samples were taken from each piglet via the orbital sinus
technique using a Monoject™ Standard Hypodermic needle 16G x 1” (Covidien™,
Mansfield, MA, USA). Serum was collected in 8.5 mL tubes (BD Vacutainer®, BD,
Franklin Lakes, NJ, USA) and whole blood samples were collected in 6 mL tubes
containing ethylenediamine tetraacetic acid (EDTA) (BD Vacutainer®, BD, Franklin
Lakes, NJ, USA). At the second visit, 3 weeks after the first visit, the same pigs were
weighed and whole blood samples were taken using the same techniques described
above. In order to evaluate the prevalence of iron deficiency and anemia in piglets at
weaning, a classification for Hb status was determined a priori based on current
classifications found in the literature. Normal iron status was defined as a Hb value of
>110 g/L, iron deficiency was defined as a Hb value of >90 g/L but ≤110 g/L and anemia
was defined as a Hb value of ≤90 g/L.17,18 The prevalence of iron deficiency and anemia
in piglets was determined for each farm.

**Hemoglobin Measurement:**

Hemoglobin values were analyzed using two methods. On the initial sampling, 1
to 2 days prior to weaning, the whole blood samples collected from each of the piglets
were analysed at the Animal Health Laboratory (AHL) at the University of Guelph using
the ADVIA 2120/2120i Hematology system (Siemens Healthcare Diagnostics, Deerfield,
IL, USA) as per standard protocols. Briefly, the blood sample and the ADVIA 2120 HGB
reagent are mixed together in the Hb chamber of a colorimeter. The Hb reaction involves
two steps: RBCs are lysed to release Hb, and heme iron found in Hb is oxidized from the ferrous to ferric state, and then combined with cyanide in the ADVIA 2120 HGB reagent to form the product.\textsuperscript{18} Optical readings are obtained colormetrically at 546nm.\textsuperscript{19}

The second Hb measurement occurred 3 weeks after the initial visit, when the pigs were in the nursery. At this time, Hb in whole blood samples was measured using an automated hematology handheld instrument (STAT-Site\textsuperscript{0} M\textsuperscript{Hgb} meter, Boerne, Texas). The STAT-Site\textsuperscript{0} M\textsuperscript{Hgb} contains a plastic card with reagent pads for determining the concentration of Hb. This device provides measurements of blood Hb content within 30 sec after 1.5 μL of blood has been applied to the test strip. The amount of color produced from azide-methemoglobin is proportional to the concentration of Hb in the sample.\textsuperscript{20}

\textit{Hematology Measurements}

The red blood cell count (RBCC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were analyzed on whole blood samples collected at the first visit, by the AHL using the ADVIA 2120/2120i Hematology System (Siemens Healthcare Diagnostics, Deerfield, IL, USA). All of these indicators of iron status were analyzed from a single optical cytometer after the dilution of the samples with ADIVA 2120 RBC reagent. As the reaction mixture moves towards the flowcell in the optical cytometer, a laser strikes the cells and generates electronic scatter signals to measure the size, volume and internal characteristics of the cells.

To determine serum iron and total iron binding capacity (TIBC), the same whole blood samples were also analyzed by the AHL using a Roche/Hitachi cobas 6000 c501
analyzer. Briefly, iron is released from transferrin under acidic conditions. Ascorbate decreases the amount of released Fe3+ ions to Fe2+ ions, which then react with FerroZine. This reaction forms a colored complex in which TIBC is measured photometrically and is proportional to the color intensity.

**Zinc levels in the feed:**

Feed tags were collected at each farm to evaluate the concentration of zinc in the first phase nursery diets. Zinc level in the feed was categorized: nutritional dose (≤500 mg/kg), high dose (2000-3000 mg/kg) and very high dose (>3000 mg/kg).

**Statistical Analysis:**

All data were entered into an Excel spreadsheet and then imported into Stata 12 Intercooled for Windows XP (Statacorp LP, College Station, Texas, USA) for analyses. The association between Hb status (g/L) at weaning and subsequent nursery growth performance (measured as the 3-week post-weaning weight) was analyzed using mixed linear regression. The dependent variable in the model was the 3-week post-weaning body weight. The covariates that were used in the final model include: parity of the sow, age at weaning, weaning weight, and Hb status at weaning. The model was built using forward stepwise criteria and designed parsimoniously. Hemoglobin status was categorized as follows: normal Hb (>110 g/L), iron deficient (>90 g/L but ≤110 g/L) or anemic (≤90 g/L). Dam (sow) parity was categorized as: parity 1 (gilt), parity 2-5, and parity >5. The age of the piglet when they were given their iron supplementation was categorized as: ≤1 day of age, 2 to 4 days of age, and 5 to 7 days of age.
All extraneous variables were initially screened for univariable associations using linear regression and considering farm as a random effect. Univariable associations with a liberal P-value of <.2 were considered for the final model. Linearity of continuous predictor variables with 3-week post-weaning weight as the outcome variable was assessed using two methods: 1) visually using a lowess smoother (smoothed locally weighted scatter plot), and 2) using a quadratic term. Confounding was assessed throughout model building. A confounding variable was defined as a variable whose removal from the model changed the coefficient of any predictor variable by greater than 20%. Two-way interactions were generated between all extraneous variables in the initial model and were only included in the final model if they were statistically significant (P <.05). Extraneous variables were included in the final model if: 1) variables had a P <.2 in univariable analysis and then a (P <.05) in the final model, 2) if the variable was a confounder or 3) if the variable was part of a statistically significant interaction (P <.05). These variables were then assessed for collinearity using Pearson correlation analysis. Potential outliers, influential points and the model assumptions were assessed graphically.

To assess the association between various iron indicators and weaning weight (category), 8 separate linear mixed models were created. Each iron indicator (RBC, Hb, HCT, MCV, MCH, MCHC, serum iron and TIBC) was modeled separately as the dependent variable and in each of the models, the association between the size of the piglet at weaning (small, medium or large) and each iron indicator was evaluated. In these models, sex of the piglet, parity of the dam, age at weaning, weaning weight, and
the type of iron administered were modeled as fixed effects and farm was modeled as a random effect using the same methods described above for the analysis involving Hb.

To explore the association between the zinc content in-feed and anemia at 3-weeks post-weaning a mixed logistic regression model was built. For this model, iron status was dichotomized as anemic if the piglet had a Hb concentration ≤90 g/L or normal if the piglet had a Hb concentration >90 g/L. Iron status at weaning, the type of iron administered, and age at weaning were modeled as fixed effects. Confounding was assessed throughout model building as described above. Potential outliers and influential points were evaluated graphically and model diagnostics were performed.

3.4 Results

The farms enrolled varied in size from 112 to 1500 sows, with the majority of farms being farrow-to-finish and 4 being farrow-to-wean. A total of 1095 pigs were sampled with a range of 13-20 litters per farm. All male piglets enrolled in this study had been castrated. Farm-specific demographics, iron supplementation protocols, and mean growth performance values including piglet weaning weight, 3-week post-weaning weight and average daily gain (ADG) for each farm are presented in Table 3.1. Of the twenty farms sampled 60% (12/20) mixed their iron product with other pharmaceutical products such as meloxicam or penicillin.

The mean age at which piglets were initially sampled (1 to 2 days prior to weaning) was 21.8 ± 4.2 days. The mean weight of pigs at initial sampling (1 to 2 days prior to weaning) was 6.4 ± 1.8 kg. The mean weight of pigs in the small (n= 365), medium (n= 365), and large (n= 365) weight categories were 5.2 ± 1.5 kg, 6.5 ± 1.4 kg,
and 7.5 ± 1.6 kg, respectively. The prevalence of iron deficiency and anemia at 1 to 2 days prior to weaning and at 3-week post-weaning for individual farms are presented in Table 3.1. The within herd prevalence of iron deficiency and anemia at weaning ranged between 0-61% and 0-46%, respectively. The mean herd prevalence of iron deficiency and anemia at weaning were 28% and 6%, respectively. Nineteen out of the twenty (95%) farms had piglets with low Hb values (iron deficient and/or anemic) at weaning. Upon sampling at 3-weeks post-weaning, the within herd prevalence for iron deficiency and anemia ranged between 29-74% and 6-32%, respectively. The mean herd prevalence of iron deficiency and anemia at this time was 43% and 18%, respectively. From the initial sampling day to 3-weeks post-weaning, 72 (6.6%) pigs went missing. The reasons for piglet loss were not recorded but pig loss was evenly distributed amongst the farms. From the 72 pigs that were missing, 58% had normal Hb values, 35% were iron deficient and 7% were anemic at weaning.

The final model illustrating the association between Hb status at weaning and 3-week post-weaning weight is presented in Table 3.2. There were no significant interactions or confounders identified. The final model revealed that anemic piglets at weaning had a 0.82 kg lower 3-week post-weaning weight compared to piglets with normal hemoglobin values at weaning ($P<.01$). Also, anemic pigs at weaning were on average 0.69 kg lighter in weight at 3-week post-weaning compared to pigs that were classified at weaning as iron deficient ($P<.05$).

Piglets that were from sows whose parity ranged from 2-4 and >5 had higher 3-week post-weaning weights compared to piglets from gilts ($P<.05$). There was no difference found in the 3-week post-weaning weights between piglets administered an
iron dextran injection or a gleptoferron injection. Piglets weaned at an older age had a 0.12 kg higher weight at 3-weeks post-weaning ($P<.001$). Medium sized piglets at weaning had a Hb concentration 2.7 g/L higher than larger pigs at weaning ($P<.01$). Smaller sized pigs at weaning had a Hb concentration 3.4 g/L greater than large pigs at weaning ($P<.001$).

The 8 models illustrating the associations between various iron indicators with body weight category at weaning are presented in Table 3.3. The mean values for various iron indicators based on piglet weight category can be found in Table 3.4. Piglets from the large weight category had lower Hb, serum iron, HCT, MCV, MCH and MCHC values compared to piglets in the smaller weight category ($P<.05$). Total iron binding capacity values were higher in the large sized piglets compared to the small and medium sized piglets ($P<.01$). There was no difference found between each of the weight categories and RBCs.

The zinc content in feed used on the farms ranged between 250-7000 mg per kg of feed. The mixed logistic regression model created to explore the association between the zinc content in-feed and anemia at 3-weeks post weaning is presented in Table 3.5. The odds of nursery pigs being anemic was 3.4 times greater for those pigs consuming high doses of zinc in-feed compared those consuming a nutritional dose of zinc in-feed ($P<.05$). The odds of nursery pigs being anemic was 4.1 times greater for those consuming starter feeds containing very high levels of zinc compared to those consuming a nutritional dose of zinc ($P<.05$). There was no difference in the odds of anemia in pigs fed very high doses of zinc compared to pigs fed an high dose of zinc in-feed ($P>.05$). The type of iron administered was a confounder in this model. This is likely because
anemia was classified differently (dichotomized) in this model compared to categorical in the main model and because the majority of farms used iron dextran, hence this variable was included in the model.

3.5 Discussion

Despite routine iron supplementation during the first week of life, pigs with low Hb values were found at weaning on almost all farms and the prevalence of anemic pigs was greater 3 weeks after weaning. There are different reference limits reported in the literature regarding how low Hb levels can be before anemia is diagnosed.\textsuperscript{13,17,18} Wei et al.\textsuperscript{13} suggest that a hemoglobin concentration above 100 g/L is considered normal and that a Hb concentration below 60 g/L indicates severe anemia. Anemia has also been defined as a Hb concentration below 80 g/L.\textsuperscript{12} A recent paper by Bhattarai and Nielsen\textsuperscript{18} used a Hb concentration below 110 g/L as indicative of iron deficiency and a Hb concentration below 90 g/L as indicative of anaemia. Being able to distinguish low iron prior to evidence of clinical anemia is a useful concept for practitioners who are monitoring the effectiveness of an iron supplementation program and for this reason we chose to use the Bhattarai and Nielsen categories to assess Hb concentrations.

Although Hb status is the most frequently used parameter for evaluating iron deficiency and anemia in swine, it is possible that there are other blood parameters that may be more sensitive in detecting the early stage of iron deficiency.\textsuperscript{21} For instance, Bhattarai and Nielsen\textsuperscript{18} were not able to find a difference in hemoglobin concentration between various piglet sizes but found that large pigs had lower serum iron and higher TIBC than other pigs indicating that iron is utilized faster in bigger piglets making them prone to iron deficiency and also concluding that using hemoglobin as a diagnostic tool
may underestimate the iron requirements for young growing piglets. With this in mind, additional iron indicators were analyzed in this study. The results for serum iron, HCT, MCV, MCH and MCHC in each pig weight category at weaning agreed with the results of the hemoglobin measurements for assessing iron status in this study. Since Hb can be easily and inexpensively measured using hand-held instruments that can be used on-farm, these results support the continued use of Hb to monitor iron status on pig farms.

All of the measurements for iron status used in the present study indicate that the larger piglets at weaning were more likely to be iron deficient compared to the small and medium sized piglets. The data from the current study also indicate that on most farms the traditional supplementation of 200 mg of parenteral iron is insufficient to meet the needs of the large and fast growing piglets and a higher dosage of iron or a second injection of iron at a later date during the suckling period may be required.

The prevalence of anemia and iron deficiency in pigs at weaning in the current study is similar to the results from recent studies in various countries. Walsh et al. found 30% of Ontario pigs to be anemic at weaning, when assessing Hb status on a single commercial swine farm. The current study confirms that identifying anemic piglets at weaning is not uncommon on Ontario pig farms. There are other possible reasons in addition to greater nutritional requirements for fast growing pigs which might explain why anemic and iron deficient piglets are present at weaning. One possible reason is human error during the administration of the iron supplementation e.g. some piglets are missed during the process or iron is given to older pigs. It is also possible that there could be injection site leakage resulting in dose variation when administering the iron during processing, thereby having some pigs more at risk for anemia due to not receiving a full
dose of iron product. In this study, on 60% of the participating farms, an iron product was used that had been mixed on-farm with penicillin or meloxicam. This is surprising because “mixing two or more medications in syringe for delivery to animals is a form of compounding and is not permitted” according to the Canadian Quality Assurance program (CQA® Producer Manual, Version 2.1, D4-6, 2007) and Health Canada's policy on drug compounding in human and veterinary medicine (Policy on Manufacturing and Compounding Drug Products in Canada POL-0051, 2009). To the authors knowledge it is unknown what effect, if any, that compounding pharmaceuticals with iron products may have on the uptake of iron by the piglet. Nevertheless, if iron is mixed with other products there is a risk that one of the products settles and when the compounded product is drawn into a syringe the proportion of iron may not be the expected concentration, so that some piglets are under dosed while others receive a high dose.

The rapid growth rate of modern piglets is a concern because iron requirements are likely increased. The large sized piglets in this study had lower Hb concentrations at weaning compared to the smaller and medium sized piglets. Jolliff and Mahan \(^1\) also found that heavier piglet weaning weight was associated with decreased Hb and HCT values. The reason for this may be explained by the fact that each piglet receives a fixed amount of iron from maternal stores.\(^7\) Smaller piglets will have less blood volume, thereby having a more proportional Hb concentration for optimum synthesis. Larger piglets have a larger blood volume, therefore diluting Hb and increasing their iron requirements making them more susceptible to iron deficiency and anemia. In this study the larger pigs selected at weaning had lower Hb values compared to the smaller and medium sized pigs indicating that a single 200 mg IM injection of either iron dextran or
gleptoferron is not sufficient in preventing iron deficiency and anemia in some rapidly growing pigs.

The timing of the iron injection, specifically the age of the pig when iron is administered, is also important to consider when assessing Hb status at weaning. The producer from each participating farm completed a questionnaire and indicated the piglets' age at which iron was administered. However, in reality there was likely minor variation on farm. It is unknown how stringently the producers followed their own iron supplementation protocol, since it may not always be possible to administer iron on the same day of age after every litter of piglets is born. This limitation of the study may have introduced some misclassification bias to the variable day of iron administration. The literature indicates that parenteral iron supplementation within the first 3-4 days of age can prevent suckling pigs from anemia. In this study, the range of age at which suckling pigs were administered iron was from within the first 24 hours up until 7 days of age, and the majority of producers reported that they administered iron within 3 to 4 days of age. This may explain why this study did not find an association with timing of administration of iron supplementation since the majority of farms administered iron products by the time pigs were 7 days of age.

The type of iron administration, either oral or parenteral, and the dose is another important consideration when assessing Hb at weaning. Among the 20 participating farms, all farms supplemented their piglets with an IM injection of either a gleptoferron or iron dextran product. The majority of farms used iron dextran and no difference was found between farms using gleptoferron or iron dextran. This finding is consistent with other studies that have reported no difference between gleptoferron and iron dextran in
preventing iron deficiency and anemia.\textsuperscript{26, 27} This suggests that iron from both gleptoferron and iron dextran is utilized with comparable efficacy for hemoglobin synthesis and iron storage in young growing pigs. On most farms pigs have access to creep feed containing iron. It was not possible in the current study to determine whether the intake of creep feed contributed to the piglet’s iron status.

A different test method was used to assess Hb status at 3-weeks post-weaning compared to the test used to assess the suckling piglets. Hemoglobin status has been evaluated using a handheld device on farm.\textsuperscript{28-30} During the second farm visit to each participating farm, Hb measurements were evaluated using a STAT-Site\textsuperscript{®} MHgb handheld meter. This convenient handheld meter can be utilized while on-farm to evaluate analytical results more rapidly compared to submitting samples to a laboratory service. A limitation to using this particular handheld device is that it has not been used to assess Hb content in swine. However, this device has been tested on humans and has a 0.93 correlation coefficient when compared to standard laboratory testing.\textsuperscript{30} Another handheld meter, the HemoCue\textsuperscript{®}, is a similar device that has been used to assess Hb measurements in both humans and swine.\textsuperscript{28, 29} This device measures Hb content via the conjugation of free Hb to azidemethaemoglobin and photometrically measured at 570 nm, whereas the STAT-Site\textsuperscript{®} MHgb meter uses the same method but is photometrically measured at 565 nm.\textsuperscript{29, 30} Kutter et al.\textsuperscript{28} reported good agreement after assessing the HemoCue\textsuperscript{®} meter with standardized Hb laboratory measurements. The sensitivity and specificity of the HemoCue\textsuperscript{®} meter were 97\% and 100\%, respectively.\textsuperscript{31}

Similar to suckling piglets, nursery pigs also grow rapidly resulting in a rapid increase in blood volume and a high nutritional iron requirement.\textsuperscript{32} The decrease in Hb
concentrations when the nursery pigs were sampled using the STAT-Site® \( M^{\text{Hgb}} \) meter suggests that hemoglobin synthesis may not increase proportionally as the rapidly growing nursery pigs increase in weight and blood volume.\(^{11}\) There are a few possible reasons why iron deficiency and anemia continued 3-weeks post-weaning. Firstly, the types of iron used in the nursery rations may have varied between farms, some being more readily absorbed than others. Although the iron concentrations varied in the nursery rations, all concentrations were well over NRC requirements. The stress of weaning is also associated with a reduction in feed consumption, which may play a role in iron deficiency and anemia post-weaning.\(^{33}\) In a previous study, it was suggested that intestinal regulation of iron absorption might not be entirely functional within the first few weeks post-weaning.\(^{34}\) Additionally, Hansen et al.\(^{32}\) found that mRNA levels of intestinal iron transcript levels of divalent metal transporter 1 (DMT1) are not up-regulated in the pigs until they reach between 26 and 47 days of age and noted that this period of time was a critical developmental period for rapidly growing pigs. In addition, iron absorption is controlled by hepcidin antimicrobial peptide (HAMP), which is derived from the liver and is produced in response to high levels of iron.\(^{34}\) HAMP binds to ferroportin (an iron exporter) on cell surfaces and degrades the cells.\(^{32}\) Hansen et al.\(^{32}\) also conclude that HAMP-mediated iron homeostasis is likely not fully functional in newly weaned pigs and that these pigs are not able to properly react to changes in dietary iron.

In this study piglets that were anemic grew slower, and while not measured in this study, may also be more susceptible to disease due to having a compromised immune system that is commonly associated with anemia.\(^{35}\) This may not be initially obvious to
the producer because these piglets are often the biggest at weaning, however, this can lead to economic issues in the future if mortality and morbidity rates are elevated due to an underlying anemia.

The solution to preventing iron deficiency in piglets at the time of weaning needs to be investigated. There is a danger of iron toxicity and a concern of increasing bacteremia if the dosage of iron is increased in the first few days of life. A second injection close to or at the time of weaning may be a good way to provide sufficient iron to the pig to meet the nutritional demands of the early weanling stage without increasing the risk of toxicity but a second injection is associated with an increased labor cost. There may be an economic benefit for adding a second injection to overall benefit of piglet health and performance that might outweigh the cost of labour, but this needs to be assessed on individual farms and warrants further study.

Iron deficiency and anemia were associated with decreased growth rate (poor nursery performance) in this study. Pigs that were iron deficient and anemic at weaning had a reduction in 3-week nursery bodyweight when compared to pigs with normal hemoglobin levels and this is consistent with other reports. Schrama et al. found that piglets with low hemoglobin values had lower ADG compared to piglets with higher hemoglobin levels. Gentry et al. also found that pigs with higher hemoglobin levels at weaning had greater ADG and higher feed intake post-weaning. These results are comparable to the current study, since 3-week post-weaning weight was positively associated with Hb status at weaning.

In order to investigate the effects of hemoglobin status on post-weaning performance, various farm management protocols were accounted for in the analyses.
Piglet weaning weight and age were controlled for because they are both significant contributors to 3-week growth performance in nursery pigs. Piglet weight at weaning was positively associated with 3-week post-weaning weight in the nursery barn, which is consistent with previous studies.\textsuperscript{38,39} Piglets that were weaned at an older age reached a greater 3-week post-weaning weight compared to piglets weaned at an earlier age.

Both litter size and sex were not included in the final model because these variables had no significant association on 3-week post-weaning weight on univariable analysis. The parity of sow was included in the final model as there was a statistically significant association found with 3-week post-weaning weight in univariable analysis as well as in the final model. In a previous study conducted by Smith et al,\textsuperscript{40} piglets born to primiparous sows had a slower growth rate compared to piglets from sows with a higher parity. The same was found in this current study, as piglets from higher parity sows had higher 3-week post-weaning body weights compared to primiparous sows.

High concentrations of zinc-oxide ($\geq 2000$ mg/kg) in-feed are commonly used therapeutically in starter rations to control post-weaning \textit{E. coli} diarrhea.\textsuperscript{41-43} A possible reason why nursery pigs had greater odds of being anemic when consuming high and very high doses of zinc in-feed compared to a nutritional dose of zinc is because high doses of zinc interferes with the conversion of iron into ferritin.\textsuperscript{41} It is also possible that both high and very high levels of zinc in-feed may increase the iron requirement in young growing pigs due to decreasing the life span of red blood cells.\textsuperscript{41} Further, zinc, copper and iron are metals that interact and may present competitive inhibition of transport and bioavailability.\textsuperscript{44,45} Therefore, the interactions between metals such as copper and zinc may affect iron absorption. In aqueous solutions and at higher doses, competition
between metals with similar properties can occur.\textsuperscript{46} There are many inhibitory interactions between these metals that could occur when high doses of a certain metal are given.\textsuperscript{46} In a competition study, when the concentration of copper and zinc were increased iron uptake was decreased.\textsuperscript{46} In another study, zinc status influenced iron uptake, indicating that divalent metal transporter 1 (DMT1) may not simultaneously transport iron and zinc.\textsuperscript{47,48} A limitation to this study is that the length of time in which the pigs were fed the nursery rations with the specific concentration of zinc oxide were not measured. The use of high concentrations of zinc in-feed are likely to interfere with iron absorption, and thus should be looked into further in future studies in more detail.

Copper is another mineral that could have an effect on iron deficiency and anemia in young growing pigs. However, due to the lack of variability of copper content in-feed between the participating farms in this study, copper could not be analyzed. Both copper and zinc are heavy metals that are being used therapeutically in-feed.

In summary, this study identified iron deficiency and anemia in newly weaned pigs and in pigs that were 3 weeks after weaning. There was evidence that anemia causes a negative impact on growth performance post-weaning. The widespread prevalence of iron deficiency and anemia on almost all farms in this study indicates that iron status should be monitored on all farms and supplementation programs accessed.

3.6 Implications

- Supplementation of 200 mg injectable iron was not sufficient to meet iron requirements of large fast growing suckling pigs
- The prevalence of iron deficiency and anemia increased 3 weeks into the nursery
• Iron deficiency and anemia is negatively associated with post-weaning growth
• High levels (>2000 mg/kg) of zinc oxide in starter diets might be a contributory factor for the presence of anemia in weaned pigs

Acknowledgements
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Conflict of interest
None reported

Disclaimer
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3.7 References


31. STAT Site® M\textsuperscript{Hgb} [Whole Blood Hemoglobin Test Cards] Boerne, Texas: Stanbio Laboratory; 2012.


Table 3.1: Summary of farm production parameters, iron supplementation protocols, and iron status of piglets at the time of weaning and at 3 weeks post-weaning from 20 commercial swine farms in Ontario

<table>
<thead>
<tr>
<th>Farm (no. of sows)</th>
<th>No. of pigs at weaning (no. of litters)</th>
<th>Age (d)</th>
<th>Mean Weight (kg)</th>
<th>Iron Status†</th>
<th>% affected at weaning (n)</th>
<th>% affected at 3 weeks post-weaning (n)</th>
<th>ADG (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1† (600)</td>
<td>57 (19)</td>
<td>&lt;1††</td>
<td>21.4 (±2.2)</td>
<td>3 weeks post-weaning (±SD)</td>
<td>12.0 (±2.9)</td>
<td>35 (20) 61 (35) 4 (2) 63 (35) 30 (17) 7 (4)</td>
<td>0.25 (±0.06)</td>
</tr>
<tr>
<td>2* (1400)</td>
<td>60 (20)</td>
<td>2-4</td>
<td>19.2 (±0.9)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>17.4 (±2.9)</td>
<td>63 (38) 37 (22) 0 (0) 62 (37) 28 (17) 10 (6)</td>
<td>0.34 (±0.08)</td>
</tr>
<tr>
<td>3† (500)</td>
<td>60 (20)</td>
<td>5-7</td>
<td>27.9 (±2.8)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>17.4 (±2.9)</td>
<td>72 (43) 27 (16) 1 (1) 62 (37) 28 (17) 10 (6)</td>
<td>0.40 (±0.09)</td>
</tr>
<tr>
<td>4† (300)</td>
<td>60 (20)</td>
<td>5-7</td>
<td>20.9 (±1.7)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>11.5 (±2.3)</td>
<td>80 (48) 12 (7) 8 (5) 62 (34) 28 (14) 23 (14)</td>
<td>0.27 (±0.06)</td>
</tr>
<tr>
<td>5† (250)</td>
<td>51 (17)</td>
<td>5-7</td>
<td>24.5 (±1.3)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>12.7 (±2.7)</td>
<td>37 (19) 39 (20) 24 (12) 19 (9) 75 (35) 6 (3)</td>
<td>0.29 (±0.09)</td>
</tr>
<tr>
<td>6† (112)</td>
<td>48 (16)</td>
<td>5-7††</td>
<td>26.2 (±2.7)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>11.5 (±3.1)</td>
<td>6 (3) 48 (23) 46 (22) 6 (13) 64 (30) 4 (4)</td>
<td>0.23 (±0.09)</td>
</tr>
<tr>
<td>7†* (1000)</td>
<td>60 (20)</td>
<td>&lt;1</td>
<td>21.0 (±1.7)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>12.6 (±1.9)</td>
<td>30 (18) 58 (35) 12 (7) 59 (35) 29 (17) 12 (7)</td>
<td>0.29 (±0.06)</td>
</tr>
<tr>
<td>8† (850)</td>
<td>60 (20)</td>
<td>2-4††</td>
<td>18.7 (±1.3)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>9.3 (±2.6)</td>
<td>70 (42) 25 (15) 5 (3) 35 (21) 33 (20) 32 (19)</td>
<td>0.16 (±0.08)</td>
</tr>
<tr>
<td>9† (140)</td>
<td>39 (13)</td>
<td>&lt;1</td>
<td>29.5 (±3.2)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>7.3 (±1.7)</td>
<td>59 (23) 33 (13) 8 (3) 26 (10) 44 (17) 30 (12)</td>
<td>0.10 (±0.06)</td>
</tr>
<tr>
<td>10† (1250)</td>
<td>60 (20)</td>
<td>2-4</td>
<td>25.3 (±1.6)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>14.4 (±2.8)</td>
<td>83 (50) 17 (10) 0 (0) 27 (16) 42 (25) 31 (18)</td>
<td>0.36 (±0.08)</td>
</tr>
<tr>
<td>11† (130)</td>
<td>39 (13)</td>
<td>2-4</td>
<td>25.5 (±3.5)</td>
<td>Normal† Deficient‡ Anemic§</td>
<td>14.5 (±3.2)</td>
<td>100 (39) 0 (0) 0 (0) 28 (11) 46 (18) 26 (10)</td>
<td>0.31 (±0.16)</td>
</tr>
</tbody>
</table>
### Hemoglobin Results

| Farm       | No. | Age | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final | Initial | Final |
|------------|-----|-----|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|---------|-------|
| 12"       | 60  | 2-4 | 17.5    | 6.0   | 11.0    | 80    | 20      | 0     | 13      | 51    | 36      | 0.24  |
| (640)     |     |     | (+1.0)  | (+1.2) | (+2.2)  | (48)  | (12)    | (0)   | (8)     | (30)  | (21)    | (+0.08)|
| 13"       | 60  | 2-4 | 19.9    | 5.6   | 11.1    | 3     | 0       | 28    | 45      | 27    | 0.26    |
| (1500)    |     |     | (+0.7)  | (+1.4) | (+2.1)  | (58)  | (2)     | (0)   | (17)    | (27)  | (16)    | (+0.05)|
| 14†        | 57  | 2-4 | 17.1    | 5.2   | 8.6     | 77    | 23      | 0     | 51      | 37    | 12      | 0.17  |
| (600)     |     |     | (+1.3)  | (+1.2) | (+1.6)  | (44)  | (13)    | (0)   | (29)    | (21)  | (7)     | (+0.04)|
| 15†        | 60  | <1  | 21.1    | 6.8   | 13.3    | 70    | 30      | 0     | 48      | 32    | 20      | 0.22  |
| (535)     |     |     | (+2.7)  | (+1.7) | (+3.0)  | (42)  | (18)    | (0)   | (29)    | (19)  | (12)    | (+0.06)|
| 16†        | 60  | <1  | 18.0    | 5.1   | 8.7     | 92    | 8       | 0     | 34      | 53    | 13      | 0.16  |
| (250)     |     |     | (+1.7)  | (+1.2) | (+2.1)  | (55)  | (5)     | (0)   | (20)    | (32)  | (8)     | (+0.06)|
| 17‡        | 42  | 2-4 | 18.3    | 5.3   | 12.8    | 48    | 50      | 2     | 37      | 49    | 14      | 0.36  |
| (85)      |     |     | (+4.3)  | (+1.5) | (+3.4)  | (20)  | (21)    | (1)   | (15)    | (20)  | (6)     | (+0.10)|
| 18‡        | 60  | 2-4 | 20.8    | 6.4   | 11.2    | 98    | 2       | 0     | 40      | 35    | 25      | 0.23  |
| (420)     |     |     | (+1.4)  | (+1.4) | (+1.7)  | (59)  | (1)     | (0)   | (24)    | (21)  | (15)    | (+0.06)|
| 19‡        | 42  | 2-4 | 21.7    | 6.0   | 11.6    | 31    | 52      | 17    | 37      | 56    | 7       | 0.26  |
| (262)     |     |     | (+4.0)  | (+1.2) | (+2.1)  | (13)  | (22)    | (7)   | (15)    | (23)  | (3)     | (+0.07)|
| 20‡        | 60  | 5-7† | 26.4   | 8.4   | 14.8    | 77    | 23      | 0     | 75      | 24    | 1       | 0.30  |
| (650)     |     |     | (+2.8)  | (+1.5) | (+2.4)  | (46)  | (14)    | (0)   | (44)    | (14)  | (1)     | (+0.07)|
| **TOTAL** | 54.8|     | 21.8    | 6.4   | 12.0    | 66    | 28      | 6     | 39      | 43    | 18      | 0.26  |
| (573)     | (19.9)|     | (+4.2)  | (+1.8) | (+3.4)  | (728) | (304)   | (63)  | (400)   | (437) | (186)   | (+0.11)|

* At weaning hemoglobin was measured at the Animal Health Laboratory and 3-week post-weaning hemoglobin was measured using the STAT-Site® M<sub>Hgb</sub> meter (Boerne, Texas) hand held meter

† Normal Hb = (>110g/L)

‡ Iron deficient = (≤110g/L but >90g/L)

§ Anemic = (≤90g/L)

¶ Farrow-to-finish farm

** Farrow-to-wean farm

†† Farm used gleptoferron product. All other farms used iron dextran product

TOTAL = mean of the parameter for all 20 farms enrolled

SD = standard deviation

ADG = average daily gain was calculated by (3-week post weaning weight – weaning weight)/ # of days between each visit
N/A = not available due to lost samples
Table 3.2: The final model* illustrating the effect of iron deficiency and/or anemia at weaning on 3-week post-weaning body weight (kg)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient†</th>
<th>SE</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin status‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron deficient§</td>
<td>-0.133</td>
<td>0.129</td>
<td>-0.385, 0.120</td>
<td>0.304</td>
</tr>
<tr>
<td>Anemic¶</td>
<td>-0.820</td>
<td>0.259</td>
<td>-1.327, -0.313</td>
<td>0.002</td>
</tr>
<tr>
<td>Weight at weaning</td>
<td>1.250</td>
<td>0.037</td>
<td>1.177, 1.323</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sow Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-5</td>
<td>0.343</td>
<td>0.157</td>
<td>0.035, 0.651</td>
<td>0.029</td>
</tr>
<tr>
<td>&gt;5</td>
<td>0.503</td>
<td>0.187</td>
<td>0.137, 0.869</td>
<td>0.007</td>
</tr>
<tr>
<td>Age at weaning</td>
<td>0.118</td>
<td>0.023</td>
<td>0.072, 0.163</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Mixed linear regression with farm modeled as random effect
† Coefficients represent the change in three week post-weaning weight (kg) if the variable is increased by one unit or compared to its referent category i.e. piglets that were anemic 1-2 days prior to weaning had a 0.82 kg reduction in their three week post-weaning weight compared to piglets with normal hemoglobin values.
‡Referent is normal hemoglobin (>110 g/L)
§Iron deficient (>90 g/L but ≤110 g/L)
¶Anemic (≤90 g/L)
**Referent is parity 1 (gilts)
SE = standard error
CI = confidence interval
Table 3.3: Eight individual models* illustrating the associations between various iron status indicators and the body weight category at 1 to 2 days prior to weaning from 1095 piglets from 20 Ontario commercial swine farms

<table>
<thead>
<tr>
<th>Iron status indicator as the dependent variable</th>
<th>Weight† Category (kg)</th>
<th>Coefficient‡</th>
<th>SE</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>Small</td>
<td>3.434</td>
<td>0.923</td>
<td>1.625, 5.243</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>2.700</td>
<td>0.923</td>
<td>0.891, 4.510</td>
<td>.003</td>
</tr>
<tr>
<td>Serum Iron (umol/L)</td>
<td>Small</td>
<td>6.952</td>
<td>0.820</td>
<td>5.345, 8.560</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>3.411</td>
<td>0.820</td>
<td>1.803, 5.019</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>Small</td>
<td>0.007</td>
<td>0.003</td>
<td>0.001, 0.013</td>
<td>.019</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.007</td>
<td>0.003</td>
<td>0.001, 0.013</td>
<td>.018</td>
</tr>
<tr>
<td>Mean corpuscular volume (10^-12 L)</td>
<td>Small</td>
<td>2.247</td>
<td>0.371</td>
<td>1.521, 2.974</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.279</td>
<td>0.371</td>
<td>0.552, 2.005</td>
<td>.001</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (10^-12 g)</td>
<td>Small</td>
<td>0.914</td>
<td>0.153</td>
<td>0.679, 1.148</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>0.505</td>
<td>0.182</td>
<td>0.271, 0.740</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (g/L)</td>
<td>Small</td>
<td>3.483</td>
<td>0.690</td>
<td>2.130, 4.835</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>1.552</td>
<td>0.690</td>
<td>0.200, 2.905</td>
<td>.025</td>
</tr>
<tr>
<td>Total iron binding capacity (umol/L)</td>
<td>Small</td>
<td>-14.069</td>
<td>1.375</td>
<td>-16.763, -11.374</td>
<td>&lt;.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>-5.305</td>
<td>1.375</td>
<td>-8.000, -2.609</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Red blood cells (10^12/L)</td>
<td>Small</td>
<td>-0.085</td>
<td>0.050</td>
<td>-0.183, 0.012</td>
<td>.086</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>-0.003</td>
<td>0.050</td>
<td>-0.101, 0.094</td>
<td>.945</td>
</tr>
</tbody>
</table>

* Mixed linear regression models using Stata 12 Intercooled XP for Windows (College Station, Texas, USA). For all 8 models parity of the dam, age at weaning, and weaning weight were modeled as fixed effects and farm was modeled as a random effect (coefficients not shown).
† Weight categories include small (5.2 ± 1.5 kg), medium (6.5 ± 1.4 kg) and large (7.5 ± 1.6 kg) (referent) piglets selected.
‡ Coefficient represents the change in each iron analyte 1 to 2 days prior to weaning comparing small and medium sized piglets to large piglets e.g. smaller piglets at 1 to 2 days prior to weaning have hemoglobin values 3.4 g/L higher than larger piglets 1 to 2 days prior to weaning.
SE = standard error
CI = confidence interval
Table 3.4: The mean (±SD) of various iron analytes from 1095 piglets sampled prior to weaning* from 20 Ontario commercial swine farms

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Small Pigs† (n=365)</th>
<th>Medium Pigs‡ (n=365)</th>
<th>Large Pigs§ (n=365)</th>
<th>All Pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>114.1 (±15.34)</td>
<td>113.3 (±15.75)</td>
<td>110.6 (±15.38)</td>
<td>112.7 (±15.55)</td>
</tr>
<tr>
<td>Serum iron (umol/L)</td>
<td>22.9 (±11.73)</td>
<td>19.2 (±12.94)</td>
<td>15.8 (±11.80)</td>
<td>19.3 (±12.50)</td>
</tr>
<tr>
<td>Hematocrit (L/L)</td>
<td>0.39 (±0.05)</td>
<td>0.39 (±0.05)</td>
<td>0.39 (±0.05)</td>
<td>0.38 (±0.50)</td>
</tr>
<tr>
<td>Mean corpuscular volume (10^{-15} g/L)</td>
<td>66.7 (±6.89)</td>
<td>65.6 (±7.08)</td>
<td>64.4 (±7.03)</td>
<td>65.6 (±7.06)</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (10^{-12} g/L)</td>
<td>19.8 (±2.15)</td>
<td>19.3 (±2.25)</td>
<td>18.8 (±2.26)</td>
<td>19.3 (±2.25)</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentrations (g/L)</td>
<td>295.8 (±12.46)</td>
<td>293.9 (±11.35)</td>
<td>292.3 (±12.44)</td>
<td>294.0 (±12.17)</td>
</tr>
<tr>
<td>Total iron binding capacity (umol/L)</td>
<td>74.4 (±24.89)</td>
<td>83.3 (±22.53)</td>
<td>88.5 (±22.08)</td>
<td>82.1 (±23.91)</td>
</tr>
<tr>
<td>Red blood cells (10^{12}/L)</td>
<td>5.8 (±0.78)</td>
<td>5.9 (±0.80)</td>
<td>5.9 (±0.74)</td>
<td>5.9 (±0.77)</td>
</tr>
</tbody>
</table>

* Mean age at weaning was 21.8 ± 4.2 days
† The mean weight of pigs in the small weight category was 5.2 ± 1.5 kg
‡ The mean weight of pigs in the medium weight category was 6.5 ± 1.4 kg
§ The mean weight of pigs in the large weight category was 7.5 ± 1.6 kg
Table 3.5: Model* assessing the association between zinc concentration in feed and odds of anemia in piglets at 3-weeks post-weaning

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR†</th>
<th>SE</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High dose§</td>
<td>3.436</td>
<td>1.974</td>
<td>1.114-10.595</td>
<td>0.032</td>
</tr>
<tr>
<td>Very high dose¶</td>
<td>4.092</td>
<td>2.551</td>
<td>1.206-13.889</td>
<td>0.024</td>
</tr>
<tr>
<td>Hemoglobin at weaning</td>
<td>0.983</td>
<td>0.006</td>
<td>0.971-0.996</td>
<td>0.007</td>
</tr>
<tr>
<td>Weight at weaning</td>
<td>0.918</td>
<td>0.052</td>
<td>0.822-1.025</td>
<td>0.127</td>
</tr>
<tr>
<td>Iron administered**</td>
<td>2.900</td>
<td>1.454</td>
<td>1.086-7.749</td>
<td>0.034</td>
</tr>
</tbody>
</table>

*Mixed logistic regression with farm modeled as random effect
† Represents the odds of anemia e.g. The odds of nursery pigs being anemic was 3.4 times greater for those pigs consuming high doses of zinc in-feed compared those consuming a nutritional dose of zinc in-feed.
‡ Referent is nutritional dose (<500 mg/kg)
§ High dose = (2000-3000 mg/kg)
¶ Very high dose = (>3000 mg/kg)
** Referent is gleptoferron
OR = odds ratio
SE = standard error
CI = confidence interval
CHAPTER 4: AN INVESTIGATION OF HEMATOLOGY AND BIOCHEMISTRY PARAMETERS IN ONTARIO NURSERY PIGS

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4.1 Abstract

The evaluation of hematological and biochemical parameters in swine is rarely done due to the cost of laboratory work, labour and limited availability of reference values. Biological within-herd and between-herd variation in pigs also makes it difficult for establishing reference limits. The determination of baseline reference values is critical for veterinarians and researchers for interpreting blood parameters when diagnosing, defining, and creating an action plan to treat disease(s). The objective of this paper was to provide updated reference ranges for complete blood count (CBC), iron profile and biochemistry parameters of suckling piglets in Ontario. A total of 1095 pigs were sampled from 20 Ontario swine farms for hematological evaluation. Biochemistry analysis was performed on 200 randomly selected pigs (n=10 per farm). The mean and standard deviation, range and 95% confidence interval were calculated for each biochemical and hematological parameter.

4.2 Introduction

The evaluation of complete blood count (CBC), iron profile and biochemical parameters in swine are important for many reasons. Firstly, the assessment of these parameters can be used as a component of determining the health status of a herd (1). They can also be used for identifying disease at an early stage, making corrective action and treatment possible (1, 2). The establishment of reference values can help clinicians and researchers interpret their results, which therefore helps determine disease and herd
health problems (3, 4, 5). Despite the importance of analyzing these parameters, generally hematological and biochemical assessment are rarely used in the swine industry. This may be due to the costs of labour when compared to the low economic value of an individual pig (1) or it might be the perceived notion that hematology and blood biochemistry will provide little in the way of useful information. The latter might be self-fulfilling because if veterinarians are unfamiliar with taking samples and using the results for diagnostic purposes it is possible that blood is not collected properly, the samples are of poor quality and results may be biased due to stress or excitement of the handled animal (1). There also seems to be a dearth of literature reporting reference values for commercial swine.

There are many within-herd variables that can influence both the CBC, iron profile and biochemistry parameters of pigs. These may include environmental, physiological factors including age, breed, sex, diet, housing, as well as pathogen challenge and stress (1, 3). Between-herd differences can include the same reasons mentioned above, as well as different management practices, biosecurity and health status. The CBC, iron profile and biochemistry parameters from a particular animal or herd are typically compared to reference values that have been previously developed from a similar group of animals and using similar laboratory techniques. However, due to the lack of literature for establishing reference values in weaned pigs and due to new technological advances in swine production it is important to establish new values that reflect the latest methodology as well as the recent developments in pig farming. Also, some of the earlier studies that looked at developing reference values used small sample sizes and breeds that are uncommon. Reference values are influenced by analytical
variables such as instruments and technology, time or temperature of a reaction, substrate used (5). Many laboratories have been updated with advanced automated and computerized systems to improve the overall accuracy and precision of analytical measurements. Along with laboratory advancements, on-farm production has changed from decades ago when many of the previous studies evaluated CBC and biochemical parameters. Thus, due to both advancements and improvements on-farm and within laboratories, it is necessary to periodically reassess these blood parameters to determine reference values. The objective of this study was to develop updated reference values for hematology, iron profile and biochemistry parameters of suckling piglets close to weaning age.

4.3 Materials and Methods

The animal care committee at the University of Guelph, following the guidelines of the Canadian Council for Animal Care, reviewed and approved this study (permit #2429). In a previous study conducted by Kubik et al. (6) pigs on 20 southern-Ontario swine farms were sampled. The farms varied in production type, management practice and sow-herd size but widely represented the types of herds operating in Ontario. A questionnaire (see Appendix I) was given to each producer from the participating farms to collect reference population information including age of piglets at weaning, pig flow and the size of the sow herd. Each farm was visited 1 to 2 days prior to the routine weaning day, and litters were systematically selected beginning with the initial crate in the farrowing room until a maximum of 20 litters were sampled.
From each litter, a small, medium and large size piglet were selected and ear tagged. Pigs were not included if they had any physical abnormalities such as abscess or hernia, or if they had thin body condition, or were lame (6). All participating farms were considered to have healthy pigs without any current health challenges or disease outbreaks. A technician and veterinarian took blood samples from the selected piglets by the orbital sinus bleeding technique (7) using a Monoject™ Standard Hypodermic needle 16G x 1” (Covidien™, Mansfield, MA, USA). Blood for serum biochemistry and iron profile was collected in 8.5 mL plain tubes with no additive (BD Vacutainer®, BD, Franklin Lakes, NJ, USA) and blood for hematology was collected in 6 mL tubes containing ethylenediamine tetraacetic acid (EDTA) (BD Vacutainer®, BD, Franklin Lakes, NJ, USA). The blood samples were stored and transported on ice packs.

*CBC and Iron Profile Evaluation*

The blood samples used from Kubik et al. (6) were used for CBC and to assess the iron profile of individual piglets at weaning. Within a few hours of sampling on farm, whole blood samples were sent and analyzed at the Animal Health Laboratory (AHL), University of Guelph. Standard hematology techniques using the ADVIA 2120/2120i Hematology system (Siemens Healthcare Diagnostics, Deerfield, IL, USA) were used to measure the red blood cell count (RBC), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). To measure iron, total protein, total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC), serum samples were analyzed using a Roche/Hitachi cobas 6000 c501 analyzer, as per standard protocols.
Biochemistry Evaluation

Sera samples (n=1095) were centrifuged at the AHL at the University of Guelph and then stored at -20 °C. Due to the cost associated with the biochemical analysis, 200 samples were randomly selected using a random generator in STATA 12.0 (Stata 12 Statacorp LP, College Station, Texas, USA). A total of 10 sera samples representing 10 individual pigs were randomly selected from each of the participating farms for biochemical assessment.

All biochemistry analysis was performed on the Roche Cobas 6000 c501 biochemistry analyzer (further explanations of these reactions are presented in Appendix II).

Statistical Analysis

Reference limits were evaluated using Analyze-It ® Software Ltd. (Analyze-It Version 3.0, Leeds, United Kingdom). This software program works through Microsoft Excel ®. Non-parametric methods were used since it is the recommendation with ≥120 samples (8). This method encompasses the central 95th percentiles and use the 2.5th and 97.5th fractal as the lower and upper limits, respectively (9).

Each CBC, iron and biochemical parameter was initially screened visually using histograms to assess the distribution and to identify any potential outliers. Box plots were also used to evaluate potential outliers. Box plots illustrate a sample distribution using the 25th, 50th and 75th percentiles, which are also referred to as the lower quartile (Q1), median (m or Q2) and upper quartile (Q3) (10). Horn’s algorithm using Tukey’s interquartile fences was used to identify outliers found at upper and lower extremities (9).
The interquartile range is equal to Q3 − Q1, and this range covers the central 50% of the data (10). Observations outside 1.5 IQRs may be potential outliers, and observations outside 3 IQRs are indicated as far outliers. For this study, the authors determined to remove outliers that were ≥ 3 IQRs a priori, which is the standard practice recommended (8). The mean for each hematological and biochemical parameter was calculated along with the 95% lower and upper confidence limits and the range. The 200 samples were considered as two sets of data for the analyses. The first was the full 200 samples without any outliers removed and the second was a subset of the data with outliers that were ≥ 3 IQRs removed. The first set, without any outliers removed, was included in this study to represent the true measurements of Ontario commercial pigs at weaning. The mean and standard deviation (SD), the range and the 95% confidence interval (CI) were calculated for each parameter.

4.4 Results

A total of 1095 pigs were sampled in this study for hematological parameters and iron profile. Of the 1095 total pigs, 200 pigs were randomly sampled for biochemical analysis. All male piglets enrolled in this study had been castrated. The average age at which piglets were sampled was 21.8 ± 4.2 days.

The CBC and iron parameter reference limits, mean ± SD and 95% CI’s are presented in Table 1. This includes the full data set (n=1095) and the second data set with outliers removed if ≥ 3 IQRs (n=1046). The biochemical reference limits, mean ± SD and 95% CI’s are in Table 2 containing both the full data set (n=200) and the second data set with outliers removed if ≥ 3 IQRs (n=119). There was little difference found between the
full sets of data and the data with the outliers removed. The means of each parameter remained very similar, and the confidence intervals tightened in the data sets with the outliers removed.

4.5 Discussion

The assessment of CBC, iron and biochemistry reference values in pigs at weaning are important for both veterinarians and researchers. It is important and useful to these individuals to be able to interpret parameter measurements to better understand the health status of either an individual pig or the entire herd. The interpretation of these parameters is often difficult due to the variation found within individual pigs and herds, as well as between-herd differences. There are many factors such as sex, breed, growth rate, nutritional and health status, season and stress that can affect both hematological and biochemical parameters (11). An important factor that can be attributed to within- and between-herd differences in the pigs included in this study is age. However, each farm was visited 1 to 2 days prior to weaning with similar weaning ages.

The current study sampled suckling piglets 1 to 2 days prior to weaning. There are no published references for biochemical and hematological parameters for 3-week old suckling piglets. Weaning is a stressful period for pigs, and is associated with physiological, nutritional, environmental and social challenges. Weaning is a critical stage in swine production due the rapid changes and challenges the pigs undergo. Pigs at weaning undergo rapid growth and since their immune systems are still maturing, variation may exist in hematology and biochemistry parameters. It is therefore essential to develop reference limits for this age group to diagnose disease and to enable
implementation timely intervention to prevent further issues in later phases of the barn. It is important to recognize that a few biochemical and hematological parameters in this study may have been affected by subclinical disease and marginal nutritional deficiencies. Similar Hb, Hct, MCV, MCH, MCHC and serum iron were found when comparing results to Friendship et al. (3). This may suggest that although this paper was published many years ago, there still seems to be a problem with iron deficiency and anemia in Ontario pigs at weaning. Thus, establishing benchmark and reference ranges for these parameters can help to identify subclinical disease and the health of both individual animals and the herd.

All pigs selected for sampling were considered to be healthy with no visible outward clinical signs or physical abnormalities. However, of the 1095 pigs sampled 29% were iron deficient (Hb value of >90 g/L but ≤110 g/L) and 6% were anemic (Hb value of ≤90g/L). Recent papers by Bhattarai and Nielsen (2015) and Nielsen et al. (2013) used these definitions for defining iron deficiency and anemia. The ability to differentiate low iron before clinical signs of anemia is important for assessing iron supplementation, thus the authors chose to use these definitions to assess hemoglobin. Thus, although pigs on a commercial farm may not show any clinical signs of disease, there may be subclinical disease occurring without the producer or veterinarian knowing, highlighting the importance of assessing hematology and biochemistry parameters.

When comparing biochemistry reference intervals to previous literature, most of the parameters are similar to those reported in Friendship et al. (3). When compared to Friendship et al. (3), the 200 pigs used in the current study had higher phosphorus and alkaline phosphatase. These parameters were both expected to be elevated due to ongoing
bone development since these pigs were younger in comparison to Friendship et al. (3). Also, under experimental conditions it has been found that a low level of dietary phosphorus in feed was associated with a decrease in alkaline phosphatase (14). Thus, perhaps pigs were fed diets with higher levels of phosphorus compared to pigs from Friendship et al. (3). This study also had higher levels of cholesterol, total bilirubin, conjugated bilirubin, and free bilirubin compared to Friendship et al. (3). Elevated levels of cholesterol may be the result of dietary changes over the last few decades. Changes and updates in laboratory techniques used to measure the metabolites may also explain the variation in results.

This study had slightly higher levels of albumin and lower values of urea and creatinine when compared to the results found by Friendship et al. (3). The concentration of urea is associated with protein and can be predisposed by nutrition (15). Thus, differences in protein levels in nursery diets can influence urea. However, the current study sampled pigs 1 to 2 days prior to weaning compared to pigs that were already in the nursery for a few weeks. Also, due to the much larger sample size in this current study, these differences may indicate that 1) the herds may have been suffering from subclinical disease(s) of a borderline nutritional deficiency, 2) these parameters present a wider representation of the industry and 3) simply the variation in these parameters are what we might expect in Ontario commercial hogs at weaning.

A limitation for developing reference levels for biochemical and hematological assessment is the large variation in biological differences found within pigs on a single farm as well as an even larger variation when comparing pigs from different farms. However, a strength of this study was the large sample size (n=1095) for hematology
evaluation. The current study used a sample size that is quite large (n=200), compared to Friendship et al. (3) (n=110) when evaluating biochemical parameters. Also it is important to note that the second data set with the outliers removed had very similar results when compared to the full data set. The means of the hematology parameters and iron profiles remained analogous, with the confidence intervals tightened. This suggests that the large sample size allowed for an accurate representation of hematological and biochemical reference limits found within Ontario commercial swine herds.

In summary, this study was able to develop reference limits for biochemical and hematological parameters in suckling piglets from Ontario commercial herds. With outliers removed from the data, there was little change in the means of parameters. However, since the herds selected were considered to be generally in good health and the pigs chosen were considered in good body condition, the variation in parameters might be what is expected in Ontario commercial hogs at weaning. It is possible that the variation in parameters found may indicate borderline nutritional deficiencies and subclinical disease, however for several parameters there are no other published values for this age group to compare the results to. Since weaning is a critical stage in swine production, it is important to continue to assess biochemical and hematological blood parameters in the future to obtain recent data for veterinarians and researchers to help with disease interpretation based on analysis with the most current laboratory technology.
4.6 References


Table 4.1: Complete blood count (CBC) and iron profile references values\textsuperscript{a} of Ontario pigs sampled 1 to 2 days prior to weaning\textsuperscript{b} on twenty commercial farms

<table>
<thead>
<tr>
<th>Variable</th>
<th>1095 Pigs Sampled (Full data set)</th>
<th>1046 Pigs Sampled (Outliers Removed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>WBC (x 10\textsuperscript{9}/L)</td>
<td>11.24 ± 4.47</td>
<td>4.3 – 53.9</td>
</tr>
<tr>
<td>RBC (x 10\textsuperscript{12}/L)</td>
<td>5.87 ± 0.77</td>
<td>2.0 – 8.0</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>112.7 ± 15.5</td>
<td>30.0 – 144.0</td>
</tr>
<tr>
<td>Hct (L/L)</td>
<td>0.384 ± 0.049</td>
<td>0.10 – 0.48</td>
</tr>
<tr>
<td>MCV (10\textsuperscript{-15}L)</td>
<td>66.5 ± 7.0</td>
<td>44.0 – 87.0</td>
</tr>
<tr>
<td>MCH (10\textsuperscript{12} g)</td>
<td>294.0 ± 12.2</td>
<td>262.0 – 397.0</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>18.83 ± 4.04</td>
<td>10.0 – 40.6</td>
</tr>
<tr>
<td>Platelets (x 10\textsuperscript{9}/L)</td>
<td>512.0 ± 217.5</td>
<td>49.0 – 2635.0</td>
</tr>
<tr>
<td>MPV</td>
<td>10.8 ± 2.6</td>
<td>6.6 – 22.7</td>
</tr>
<tr>
<td>Serum iron (umol/L)</td>
<td>19.3 ± 12.5</td>
<td>1.0 – 104.0</td>
</tr>
<tr>
<td>UIBC (umol/L)</td>
<td>62.8 ± 29.7</td>
<td>0.0 – 161.0</td>
</tr>
<tr>
<td>TIBC (umol/L)</td>
<td>82.1 ± 23.8</td>
<td>23.0 – 181.0</td>
</tr>
<tr>
<td>Prop Saturation (%)</td>
<td>27.4 ± 20.7</td>
<td>1.0 – 100.0</td>
</tr>
<tr>
<td>Total Protein (g/L)</td>
<td>47.3 ± 4.4</td>
<td>30.0 – 65.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reference limits were evaluated using Analyze-It \textregistered Software Ltd. (Analyze-It Version 3.0, Leeds, United Kingdom) and based on the 2.5\textsuperscript{th} and 97.5\textsuperscript{th} fractal limits using non-parametric analyses

\textsuperscript{b} Average age when piglets were sampled (1 to 2 days prior to weaning) was 21.8 ± 4.2 days

SD = standard deviation
CI = confidence interval
Table 4.2: Biochemistry reference values\(^{a}\) of Ontario pigs 1 to 2 days prior to weaning\(^{b}\) on twenty commercial farms

<table>
<thead>
<tr>
<th>Variable</th>
<th>200 Pigs Sampled (Full data set)</th>
<th>119 Pigs Sampled (Outliers Removed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.85 ± 0.19</td>
<td>1.69 – 3.22</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>3.28 ± 0.29</td>
<td>2.29 – 3.94</td>
</tr>
<tr>
<td>Magnesium (mmol/L)</td>
<td>1.15 ± 0.14</td>
<td>0.9 – 1.5</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>138.4 ± 6.1</td>
<td>94.0 – 150.0</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>5.04 ± 0.52</td>
<td>3.6 – 7.2</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>97.2 ± 4.7</td>
<td>64.0 – 106.0</td>
</tr>
<tr>
<td>Carbon Dioxide (mmol/L)</td>
<td>25.4 ± 2.8</td>
<td>18.0 – 32.0</td>
</tr>
<tr>
<td>Anion Gap (mmol/L)</td>
<td>20.9 ± 3.0</td>
<td>15.0 – 29.0</td>
</tr>
<tr>
<td>NAK ratio (mmol/L)</td>
<td>27.7 ± 2.9</td>
<td>18.0 – 36.0</td>
</tr>
<tr>
<td>Total Protein (g/L)</td>
<td>48.1 ± 4.0</td>
<td>31.0 – 61.0</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>36.9 ± 5.0</td>
<td>17.0 – 46.0</td>
</tr>
<tr>
<td>Globulin (g/L)</td>
<td>11.1 ± 4.1</td>
<td>4.0 – 33.0</td>
</tr>
<tr>
<td>AG ratio</td>
<td>3.8 ± 1.6</td>
<td>0.67 – 10.50</td>
</tr>
<tr>
<td>Urea</td>
<td>2.51 ± 1.15</td>
<td>0.7 – 9.3</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>88.8 ± 17.4</td>
<td>36.0 – 141.0</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.49 ± 0.87</td>
<td>1.6 – 8.9</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.7 ± 1.5</td>
<td>1.59 – 9.76</td>
</tr>
<tr>
<td>Total Bilirubin (umol/L)</td>
<td>6.0 ± 2.7</td>
<td>1.0 – 22.0</td>
</tr>
<tr>
<td>Conjugated Bilirubin (mmol/L)</td>
<td>2.9 ± 1.3</td>
<td>0.0 – 10.0</td>
</tr>
<tr>
<td>Free Bilirubin (mmol/L)</td>
<td>3.1 ± 2.0</td>
<td>0.0 – 15.0</td>
</tr>
<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>586.4 ± 262.8</td>
<td>160.0 – 2119.0</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>34.9 ± 13.4</td>
<td>0.0 – 75.0</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>38.0 ± 15.9</td>
<td>3.0 – 130.0</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>362.6 ± 398.1</td>
<td>111.0 – 4918.0</td>
</tr>
<tr>
<td>GLDH (U/L)</td>
<td>5.1 ± 15.6</td>
<td>0.0 – 199.0</td>
</tr>
<tr>
<td>BHBA (umol/L)</td>
<td>3.6 ± 9.2</td>
<td>0.0 – 58.0</td>
</tr>
<tr>
<td>Haptoglobin (g/L)</td>
<td>0.62 ± 0.62</td>
<td>0.24 – 3.75</td>
</tr>
<tr>
<td>Calculated Osmo. (mmol/L)</td>
<td>275.7 ± 11.8</td>
<td>188.0 – 297.0</td>
</tr>
</tbody>
</table>

Reference limits were evaluated using Analyze-It® Software Ltd. (Analyze-It Version 3.0, Leeds, United Kingdom) and based on the 2.5<sup>th</sup> and 97.5<sup>th</sup> fractal limits using non-parametric analyses.

Average age when piglets were sampled (1 to 2 days prior to weaning) was 21.8 ± 4.2 days.

SD = standard deviation
CI = confidence interval
CHAPTER 5: CONCLUSION

5.1 Research Summary and Conclusions

Research work described in this thesis evaluated various hematological and biochemical parameters of Ontario commercial pigs to assess the health status of pigs close to weaning age and nursery pigs. As mentioned, weaning piglets in a commercial setting is an abrupt event in which piglets face new challenges and stressors that occur simultaneously (1). Weaning is often associated with a reduction in feed consumption (2), growth performance, and intestinal functionality (3, 4). Although this reduced performance is common post-weaning, most pigs do recover within a few days. However, there remains a subgroup of pigs that do not overcome these new challenges of weaning and often do not thrive in the new setting. This subgroup of pigs can have high morbidity or mortality rates due to post-weaning anorexia (5).

The subgroup of piglets that do not thrive post-weaning and do not transition onto feed was the focus of Chapter Two. The purpose of this chapter was to evaluate beta-hydroxybutyrate (BHB), a serum ketone in pigs 4-7 days post-weaning to gain a better understanding of the role of post-weaning anorexia in pigs affected with periweaning failure to thrive syndrome (PFTS). A main clinical sign of PFTS and pigs that are off-feed is excessive, repetitive oral behavior, referred to as oral chomping (6). This study was able to find a significant difference in serum BHB between thin pigs (suspected to be anorexic), pigs that displayed excessive oral chomping, and healthy, non-chomping pen mates using both standard laboratory serum testing assay, Rx Monza, and a pen-side handheld ketone meter, Abbot Precision Xtra. The ability to distinguish differences in serum BHB between the three piglet groups is important for determining a benchmark for normal and high BHB levels in
newly weaned pigs. This is substantial because chomping is a predominate clinical sign of PFTS. Since there are currently no known diagnostic results, etiological factors or treatments for PFTS, the ability to identify pigs that are ketotic and suspected to be anorexic in the early stage of PFTS will further help producers to intervene in the earlier stages of the syndrome. Also, one of the farms sampled was affected with swine influenza and the serum BHB levels from these pigs were the highest compared to all other participating farms in the study. Thus, evaluating serum BHB in future studies could be important for early identification of other diseases including PFTS and porcine reproductive and respiratory syndrome (PRRS) since pigs that are affected may initially reduce or stop feed-intake before other clinical signs appear. This will allow for herd monitoring at an early stage after weaning to detect if there is any subclinical diseases occurring prior to clinical signs. The early identification of any onset of disease could prompt the necessary treatments before the problem is worsened. Another important finding in Chapter Two was that the Precision Xtra handheld ketone meter was highly correlated with the Rx Monza serum testing assay. This convenient tool can be used on farm to diagnose ketosis. Since there are no current studies published that have evaluated serum BHB in newly weaned pigs, future studies could be beneficial to develop a standard cut-off value for diagnosing ketosis. This can benefit producers since the handheld meter will allow for them to conduct a blood test to identify anorexic pigs in the early stages of the problem without having to spend time looking for oral chomping among pigs or if they are unable to correctly identify anorexic pigs.

Chapter Three evaluated iron supplementation protocols in Ontario commercial herds. Iron supplementation is a routine procedure carried out on farms, however the iron status of piglets is rarely evaluated. There have been many improvements and updates in management
practices since the standardized protocol was first made. Along with updated management, modern sow lines farrow larger litters and piglets grow at a faster rate than previous decades (7, 8). It is therefore important to evaluate whether the standardized protocol is still adequate in preventing iron deficiency and anemia in modern piglets. At weaning, the between herd prevalence of iron deficiency and anemia at weaning were 28% and 6%, respectively. The between herd prevalence of iron deficiency and anemia 3-week post-weaning increased to 43% and 18%, respectively. While in the nursery stage, pigs grow rapidly resulting in a rapid increase in blood volume and a high nutritional iron requirement (9). The increase in prevalence of iron deficiency and anemia 3-week post-weaning may be due to a variety of reasons. Firstly, the stress of weaning alone is associated with a reduction in feed-take. Also, the types of iron added to the nursery rations may have varied between farms, with some being more readily absorbed than others.

Medium and small sized piglets selected at weaning had significantly higher hemoglobin levels compared to the large pigs selected. This indicates that iron is utilized faster in larger piglets making them more prone to iron deficiency and anemia. Anemic piglets at weaning also had lower 3-week post-weaning weights compared to piglets with normal hemoglobin values at weaning (P<.01). Although all participating farms administered a 200 mg dose of injectable iron, almost all participating farms had pigs with low hemoglobin values. This indicates that a 200 mg supplementation of injectable iron was not sufficient to meet iron requirements of the large fast growing suckling pigs. Therefore, future studies should investigate whether administering a second iron injection closer to weaning could provide sufficient iron to meet physiological demands, while avoiding iron toxicity.
Modern swine practices use high levels (2000-3000 mg/kg) and very high levels (>3000 mg/kg) of zinc oxide in feed to control *Escherichia coli* diarrhea. The addition of high levels of zinc oxide to nursery rations is a common practice in Ontario (10). This study found that a very high level of zinc oxide (≥ 3000 mg/kg) in starter diets was associated with an increased likelihood of anemia post-weaning. Iron, zinc and copper are trace minerals that have similar properties. If there is an imbalance in one of these trace minerals it can have an antagonistic effect on the nutritional availability of another mineral (11). In previous literature, zinc status influenced iron uptake, indicating that divalent metal transporter 1 (DMT1) may not instantaneously transport iron and zinc (12, 13). Thus, high levels of zinc oxide in feed may interfere with iron absorption (11). Future studies, examining different levels of zinc oxide usage in feed in a controlled setting would be beneficial to better understand the role of zinc in post-weaning anemia.

Chapter Four of this thesis evaluated hematological and biochemical parameters of piglets at weaning. The objective of this chapter was to provide updated reference values for blood and biochemistry parameters of suckling piglets close to weaning age. There is no published literature assessing these parameters in suckling piglets. There are various within-herd parameters that can influence both complete blood cell counts, iron profile and biochemistry parameters. These within-herd parameters include environmental, physiological factors including pig age, breed and sex, diet, housing, as well as pathogen challenge and stress (14, 15). There is also between-herd differences in parameters that may be influenced by the same reasons mentioned above, as well as different management practices, biosecurity and herd health status. Suckling piglets close to weaning age are growing rapidly and since their immune systems are still maturing, variation exists in
hematology and biochemistry parameters. In this study there was variation in the parameters and subclinical disease(s) and nutritional deficiencies may have affected some parameters. Since there is no published literature assessing hematology and biochemistry parameters in suckling pigs close to weaning age, it is difficult to make comparisons. This study concluded that due to the large variation found between parameters, the herds may have not been disease free and that these parameters present a wider representation of the industry. The variation in these parameters also may suggest what we might expect in Ontario commercial hogs close to weaning age, since the herds selected for sampling were in good health at the time.

In all other fields of veterinary medicine hematology and blood biochemistry are used extensively as a diagnostic tool but swine veterinarians rarely take advantage of this tool. This current research has shown many advantages and benefits of doing so. For instance, without any visual clinical signs, it was found that many piglets were indeed iron deficient and anemic based on hematological analysis. When BHB was evaluated, high levels of BHB were found in thin pigs and on a farm that was affected with swine influenza. Thus this research has identified two applications: a quick method to detect ketosis and therefore one can identify pigs off feed possibly prior to the appearance of clinical signs, and the second application was to identify herds with inadequate iron supplementation. Both applications were able evaluate Hb and BHB successfully using standard laboratory assays as well as pen-side handheld meters. Iron deficiency, anemia and anorexia (ketosis) can be easily rectified but in all cases in this research the farms were unaware of the problem. This research found that there are inexpensive on-farm tests that veterinarians can easily use. The first step was to create reference values so that veterinarians can compare values in a problematic herd with
normal values. This process was started in Chapter Four for suckling pigs close to weaning, but there needs to be follow-up actions to determine reference ranges for other age groups.
5.2 References


APPENDIX I

FARM ID: ____________
DATE: ________________

Survey
(to be completed by producer at nursery site)

Swine Production Information:

Is your entire operation on a single site or multiple sites?
  o Single Site
  o Multiple Sites

How many sows do you have in your herd? ____________

What age do you wean your pigs? ____________

How often do you wean and how many pigs are weaned at one time? (ie. 200 pigs weaned once a week).
  ____________________________________________________

How many weaned pigs do you put in a nursery pen? ________

How is the nursery managed?
  o Continuous flow – rooms never emptied and pens are filled as needed
  o All-in/All-out flow – rooms emptied completely and then re-filled with pigs.
  o Other, please explain: ______________________________________________________

What temperature is set for the nursery rooms when pigs are first weaned?
  ______________

Where do you get your breeding stock (gilts)? (Check all that apply)
  o From own herd
  o From one outside source
  o From multiple outside sources

Biosecurity Information:

Does the staff have to shower before entering or exiting the barn?
  o Always
  o Sometimes
  o □ Never
Do you ever put pigs into a nursery room that contains older pigs that were held back?

- Yes
- No

Do you clean the nursery rooms with disinfectant once the pigs have left?

- Every time
- Most times
- Occasionally

How often do you clean the barn corridors with disinfectant?

- Weekly
- Monthly
- Yearly
- Never

When feed is delivered, does the feed truck driver enter the barn?

- Always
- Sometimes
- Never

Are there any cats or dogs on the farm?

- Cat(s)
- Dog(s)
- Both
- None

Do the cats or dogs ever go into any part of the nursery barn (check all that apply)?

- Office
- Feed room
- Nursery Corridors
- Nursery Room
- Never enters the barn

Have you ever seen wild birds inside your barn in the past year?

- Yes
- No

**Swine Health Management:**

Is your farm “antibiotic free” (ie. pigs are never given antibiotics)?

- Yes
- No

How long has your farm been “antibiotic free” (ie. 6 months)?

---------------------------------------------
Do you receive a premium at slaughter for raising pigs without using antibiotics?
  o Yes
  o No

If you do use antibiotics how do you administer them? (Check all that apply)
  o In feed
  o In water
  o Injection
  o Do not use antibiotics

Has your herd been affected by 'greasy pig disease' in the past 6 months?
  o Yes
  o No
  o Unsure

If yes, how long did the greasy pig disease last (ie. 4 batches / 6 weeks)?

Was treatment used to get rid of the greasy pig disease?
  o Yes
  o No
  o Unsure

How would you describe the severity of the greasy pig disease on your farm? (choose one answer)
  o Never noticed greasy pig disease.
  o It went away without treatment, and you never noticed it again.
  o It was treated once and it went away, and you never noticed it again.
  o It was treated multiple times and then it went away, and you never noticed it again.
  o It was treated multiple times but it still comes and goes.
  o It was treated multiple times but it has never went away.

What is the amount of zinc in your feed in ppm (ppm = mg/kg)?

If you are using more than 500 ppm (mg/kg) in the nursery ration, what is your reason for doing this?

What is the product name for the injectable iron that is given to your pigs?
What age do you give iron injections to pigs? ________________________________

How much iron (ml or cc) do you give to the pigs? ________________________________

Do you mix the iron with any other products (ie. antibiotics)?

__________________________
APPENDIX II

Appendix Table 1: Univariable associations* between the 3-week post-weaning weights (kg) of nursery pigs (n=1095) and farm and animal demographics and iron supplementation protocols in 20 Ontario swine farms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>P-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L) at weaning</td>
<td>-0.005</td>
<td>0.382</td>
<td>-0.019-0.007</td>
</tr>
<tr>
<td>Age (d) at weaning</td>
<td>0.331</td>
<td>&lt;0.001</td>
<td>0.286-0.376</td>
</tr>
<tr>
<td>Weight (kg) at weaning</td>
<td>1.528</td>
<td>&lt;0.001</td>
<td>1.458-1.598</td>
</tr>
<tr>
<td>Sex</td>
<td>0.552</td>
<td>0.008</td>
<td>0.147-0.957</td>
</tr>
<tr>
<td>Type of iron administered</td>
<td>1.034</td>
<td>&lt;0.001</td>
<td>0.576-1.493</td>
</tr>
<tr>
<td>Age (d) at iron administration &lt;24 h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-4 d</td>
<td>0.651</td>
<td>0.007</td>
<td>0.179-1.123</td>
</tr>
<tr>
<td>≥5 d</td>
<td>2.691</td>
<td>&lt;0.001</td>
<td>2.148-3.234</td>
</tr>
<tr>
<td>Compounding Iron</td>
<td>0.230</td>
<td>0.274</td>
<td>-0.182-0.641</td>
</tr>
<tr>
<td>Sow Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gilts (1)</td>
<td>0.589</td>
<td>0.052</td>
<td>-0.005-1.184</td>
</tr>
<tr>
<td>&gt;5</td>
<td>0.235</td>
<td>0.467</td>
<td>-0.398-0.868</td>
</tr>
<tr>
<td>Litter size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-9</td>
<td>0.242</td>
<td>0.062</td>
<td>-0.012-0.497</td>
</tr>
<tr>
<td>&gt;12</td>
<td>0.291</td>
<td>0.054</td>
<td>-0.005-0.587</td>
</tr>
</tbody>
</table>

* Mixed linear regression with farm modeled as a random effect
Appendix Table 2: Pearson Correlation coefficients between iron indicators used to assess iron status

<table>
<thead>
<tr>
<th>Iron Indicators&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hb</th>
<th>HCT</th>
<th>MCV</th>
<th>MCH</th>
<th>MCHC</th>
<th>RBC</th>
<th>Serum iron</th>
<th>TIBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HCT</td>
<td>0.96</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCV</td>
<td>0.43</td>
<td>0.44</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCH</td>
<td>0.53</td>
<td>0.45</td>
<td>0.93</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MCHC</td>
<td>0.39</td>
<td>0.13</td>
<td>0.07</td>
<td>0.38</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RBC</td>
<td>0.65</td>
<td>0.68</td>
<td>-0.34</td>
<td>-0.28</td>
<td>0.07</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum iron</td>
<td>0.23</td>
<td>0.18</td>
<td>0.24</td>
<td>0.30</td>
<td>0.20</td>
<td>-0.04</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>TIBC</td>
<td>-0.58</td>
<td>-0.56</td>
<td>-0.39</td>
<td>-0.45</td>
<td>-0.22</td>
<td>-0.26</td>
<td>-0.27</td>
<td>1.00</td>
</tr>
</tbody>
</table>

<sup>a</sup>Hb – Hemoglobin
HCT – Hematocrit
MCV – Mean corpuscular volume
MCH – Mean corpuscular hemoglobin
MCHC – Mean corpuscular hemoglobin concentration
RBC – Red blood cell
TIBC – Total iron binding capacity
APPENDIX III

All biochemistry testing was performed on the Roche Cobas 6000 c501 biochemistry analyzer. Total bilirubin, conjugated bilirubin and free bilirubin in the presence of a suitable solubilizing agent is coupled with 3,5-dichlorophenyl diazonium in a strong acidic medium (1). The colour intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically (1).

Calcium ions react with 5-nitro-5’-methyl-BAPTA (NM-BAPTA) followed by the reaction with EDTA when placed under alkaline conditions to form a complex (2). The change in absorbance between calcium ions and these reagents are directly proportional to the calcium concentration in the blood sample, which is measured photometrically (2).

Magnesium, albumin, alkaline phosphastase (ALP) and γ-glutamyltransferase (GGT) are measured using colorimetric assays. Magnesium is measured using a colorimetric endpoint method. When magnesium is placed in an alkaline solution, magnesium forms a purple complex with xylidyl blue (3). The concentration of magnesium is measured photometrically by the decrease in absorbance of xylidyl blue (3). At a pH value of 4.1, albumin can bind with bromcresol green (BCG), to form a blue-green complex (4). The intensity of the blue-green colour is directly proportional to the albumin concentration in the blood sample and is measured photometrically (4). The concentration of alkaline phosphatase (ALP) in the sample is directly proportionate to the amount of p-nitrophenol released (5). The concentration of ALP is measured by the increase in absorbance. The level of γ-glutamyltransferase (GGT) is directly proportional to the concentration of 5-amino-2-nitrobenzoate (6). The level of GGT in a sample is measured photometrically by the increase in absorbance. Cholesterol is measured using an enzymatic, colorimetric method. Cholesterol
Esters are cleaved by cholesterol esterase to form free cholesterol and fatty acids (7). Hydrogen peroxide affects the coupling of phenol and 4-aminophenazone to create a red-quinone-imine dye (7). The colour intensity of the dye is directly proportional to the cholesterol concentration in the sample.

Creatine kinase (CK), glutamate dehydrogenase (GLDH) and glucose are measured using an ultraviolet test assay. The formation of nicotinamide adenine dinucleotide phosphate (NADPH) is directly proportional to CK activity in the sample (8). The GLDH enzyme catalyzes an NADH-dependent reaction and the decrease in NADH is directly proportional to GLDH activity (9). Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate (10). The rate at which NADPH is formed is proportional to the glucose concentration found in the sample, and is measured photometrically (10).

Sodium, chloride and potassium are measured using an ion-selective electrode (ISE) (11). The ISE develops an electrical potential for the measurements of ions in solution (11). Urea is measured using a kinetic test with urease and glutamate dehydrogenase. Urea is hydrolyzed by urease to make ammonium and carbonate. In this reaction, 2-oxoglutarate reacts with ammonium when GLDH is present and NADH to produce L-glutamate (12). The rate in which NADH concentration is decreased is proportionate to the urea concentration in the sample, which is measured photometrically (12). Aspartate aminotransferase (AST) is measured by the rate of NADH oxidation (13). The disappearance of NADH in an enzymatic reaction is proportional to AST activity in the sample (13).
References


