Pre- and Post-Weaning Nutritional Strategies to Improve Growth Performance, Gut Development, and Immune Response of Pigs

by

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ABSTRACT

PRE- AND POST-WEANING NUTRITIONAL STRATEGIES TO IMPROVE GROWTH PERFORMANCE, GUT DEVELOPMENT, AND IMMUNE RESPONSE OF PIGS

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Sows' milk as a sole source of nutrients limits growth of piglets and fails to habituate piglets to pelleted, plant-based post-weaning diets. The aim of this thesis was to evaluate pre- and post-weaning nutritional strategies on piglet growth performance, gut development, and immune response. First, with respect to creep feed composition and form, piglets provided liquid milk replacer had the greatest body weight at weaning. Between weaning and one-week post-weaning, pigs provided commercial creep feed had the greatest reduction in specific activity of maltase, sucrase and lactase. When pigs were provided a high-complexity nursery diet, they had improved growth performance, feed efficiency and gut morphology, regardless of creep feed treatment. Neither creep feed nor nursery treatments had a significant impact on immune system activation. Therefore, provision of creep feed provides no additional benefit when pigs are provided a high-complexity diet after weaning.

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LIST ABBREVIATIONS

M	Absorptive Capacity
AID	Apparent Ileal Digestible
ATTD	Apparent Total Tract Digestibility
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
BW	Body Weight
Ca	Calcium
COM	Commercial Creep Feed
CF	Correction Factor
CP	Crude Protein
CD	Crypt Depth
CW	Crypt Width
°C	Degree Celsius
DHR	Dermal Hypersensitivity Response
DE	Digestible Energy
DM	Dry Matter
G:F	Gain to Feed
g	Grams
g/day	Grams Per Day
g/kg	Grams Per Kilogram
GE	Gross Energy
HIGH	High-Complexity
hr	Hour
IgG	Immunoglobulin G
IL-10	Interleukin-10
IL-6	Interleukin-6
Kcal	Kilocalories
kg	Kilogram
LMR	Liquid Milk Replacer
L	Liter
LOW	Low-Complexity
Lys	Lysine
µL	Microliter
µm	Micromolar
mg	Milligram
mL	Milliliter
mm	Millimeter
Mm	Millimolar
min	Minutes
nm	Nanometer
nmol	Nanomolar
NE	Net Energy

NO	No Creep Feed
OM	Organic Matter
OVA	Ovalbumin
PMR	Pelleted Milk Replacer
P	Phosphorus
pH	Potential of Hydrogen
P	*Probability*
SFT	Skin Fold Test
SID	Standard Ileal Digestible
SOP	Standard Operating Procedure
×g	Times Gravity
TRMT	Treatment
U	Units
VH	Villus Height
VW	Villus Width
w/v	Weight by Volume
wt/wt	Weight by Weight

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CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

The suckling phase is the period between parturition and weaning, typically lasting between 21 and 28 days for commercially reared swine. During this time, piglets are kept with littermates and the sow and primarily consume sow's milk. At weaning, pigs are separated from the sow and mixed with pigs from multiple litters within nursery pens. When piglets enter the nursery phase, they are provided a pelleted feed formulated with a combination of cereal grains (e.g., corn and wheat), animal protein sources (e.g., fishmeal, blood plasma) and milk products (e.g., whey). The nursery phase lasts 5 to 6 weeks, and typically consists of three phases, defined by changes in diet. As the nursery phases progress, animal protein sources are gradually replaced with plant protein sources (i.e. soybean meal), which reduces diet cost. This transition in ingredients is possible since pigs become better at utilizing these plant-based protein sources as they mature, and their feed intake increases, therefore reducing the need for nutrient-dense diets.

Genetic selection has resulted in hyperprolific sows with greater litter sizes and growth potential of the offspring, causing milk production to limit piglet growth (Mabry, 2015). Between 11 and 13 days of age, milk consumption alone no longer promotes maximal growth of the offspring (King, 2000; Sulabo et al., 2010a; Strathe et al., 2017). Alternative nutrient sources can be provided to improve piglet growth during the suckling period. Two alternatives include (1) creep feed and (2) milk replacer. Creep feed is the more common choice among producers as it not only serves as supplemental nutrition for suckling piglets but also acts to familiarize piglets to pelleted feed that is provided after weaning (Vila and Tummaruk, 2016). However, creep feed is consumed in variable quantities making the benefits equally variable. When consumed by piglets however, creep feed increases growth rates, and therefore weaning weights of litters (Bruininx et al., 2002; Wolter et al., 2002; van Oostrum et al., 2016). The other option is milk replacer. This is consumed much more readily by piglets since it is in a familiar liquid form, which elicits greater growth rates and weaning weights compared to litters provided a pelleted creep feed (Kim et al., 2001; Wolter et al., 2002). However, there are three major caveats of providing milk replacer. First, it is not in a pelleted form and therefore, it does not familiarize piglets to nursery diets. Second, milk replacer is more expensive versus creep feed since it is consumed in greater quantities but also requires additional labour or a liquid feeding system to provide a consistent supply to the litter. Lastly, the ingredients used in a milk replacer are dissimilar to those found in a nursery diet.

Both creep feed and milk replacer have been shown to have positive effects for pigs after weaning (e.g., reduced feeding latency, increased feed intake), but these benefits are not consistent among studies (Park et al., 2014; Agyekum et al., 2018; Heo et al., 2018). After weaning, piglets typically experience a growth lag, which occurs during a period ranging from 5-to-14 days, where the average daily gain is less than it was prior to weaning, due to low feed intake (Lallès et al., 2007). The length and severity of the growth lag has lasting effects on the pig all the way until market weight (Lallès et al., 2007).

In the past, in-feed antibiotics have been used to mitigate this growth lag; however, these have been banned due to concerns regarding antibiotic resistance (PHAC, 2021). A strategy for maintaining growth during the nursery period is to provide highly digestible diets immediately after weaning (e.g., formulated with fishmeal, whey powder, plasma meal etc.). A high-complexity diet is nutrient-dense and readily digestible by young piglets, which helps to support growth early after weaning (Skinner et al., 2014). Alternatively, low-complexity nursery diets (formulated with high inclusions of corn and soybean meal) are much cheaper but less digestible for the immature piglet gastrointestinal tract (Skinner et al., 2014; Koo et al., 2020). Research suggests that providing creep feed or a milk replacer during the suckling phase, can expedite the maturation of the gastrointestinal tract, therefore enabling piglets to utilize plant-based diets more effectively (Friend et al., 1970; Engle, 1994; Heo et al., 2018). However, limited research has been conducted on the interaction between feed provision during the suckling phase and nursery diet complexity. If piglets provided supplemental feed during the suckling phase can more effectively utilize nursery diets and are habituated to consuming a pelleted diet, perhaps the cost of creep feed could be recuperated by providing a less-expensive nursery diet without sacrificing nursery growth performance (Dunshea et al., 1999; Park et al., 2014; van Oostrum et al., 2016). This literature review will provide a summary of the knowledge regarding the effects of creep feed and milk replacer provision on piglet performance pre-and post-weaning, in addition to their effects on sow performance. Nursery diet complexity and its implications for growth will also be explored. Finally, the effects of both creep feed/milk replacer and nursery diet will be reviewed with respect to immune function and gastrointestinal physiology (morphology and enzyme activity).

1.2 CREEP FEEDING EFFECTS ON PERFORMANCE

1.2.1 Effect of Creep Feeding on Sow Performance

Milk yield of a sow is the primary source of nutrients for the offspring and, if it does not meet requirements, it can limit piglet growth (Wolter et al., 2002). Between 11 and 13 days of age, the sow's ability to produce milk can no longer support maximal piglet growth rates (Strathe et al., 2017) and this becomes even more limiting the longer the lactation period (Vila and Tummaruk, 2016). Modeling reveals that neonatal piglets can achieve growth rates up to 450 g/day (King, 2000); however, to achieve this growth rate, each piglet would require 2 kg of sow's milk per day (King, 2000). Modern sows can produce only up to 1 kg of milk per piglet per day, in ideal conditions (King, 2000; Miller et al., 2012).

Genetic selection for larger litter sizes and faster growth rates of progeny means that the milk requirements for the litter are also increased (Novotni-Dankó et al., 2015). However, the feed intake capacity of the sow is insufficient to supply the energy and nutrients required to maintain such levels of milk production (Strathe et al., 2017). Thus, the sow begins to deplete body protein and fat stores to support milk production (Swick and Benevenga, 1977; Strathe et al., 2017). Extreme body weight, fat or protein store losses during lactation cause a reduction in follicular and oocyte development and prolong the interval between weaning and estrus for sows (Schenkel et al., 2010). Primiparous sows lose more body weight and backfat than multiparous sows throughout lactation due to increased maternal energy demands and reduced gut capacity and feed intake (Esbenshade et al., 1986; Strathe et al., 2017; Jin et al., 2018). Poor body condition scores result in higher incidence of anestrus and can result in pre-mature culling (Esbenshade et al., 1986). It is estimated that a sow must complete between 3 and 4 pregnancies before becoming profitable (Stalder et al., 2003). Therefore, implementing strategies to minimize sow body weight losses during lactation will improve sow longevity.

Creep feed is proposed to assist in reducing the energy deficit of lactating sows by reducing the amount of milk necessary to maximize piglet growth rates, since the piglets have access to supplemental nutrients and energy. Novotni-Dankó et al. (2015) found that the provision of a milk replacer minimized the amount of sow backfat lost during lactation, but other reproduction parameters (i.e. lactation feed intake or estrus interval) were not analyzed. However, there were no differences in feed intake of the sow or change in body weight during lactation when piglets were provided milk replacer (Wolter et al. 2002) or a pelleted creep feed (Sulabo et al. 2010). The difference in results is likely attributed to age of weaning, since the piglets in Novotni-Dankó et al. (2015) were weaned at 28 days of age, whereas the piglets in Wolter et al., (2002) and Sulabo et al., (2010b) studies were weaned at 21 days of age. Creep feed or milk replacer intakes are relatively low for young pigs, but feed intake for the last 7 days prior to weaning account for more than half of all feed consumed, regardless if pigs are weaned at 21 or 28 days of age (Fraser et al., 1994; Bruininx et al., 2004; Pluske et al., 2007; Sulabo et al., 2008). When piglets are weaned at a later age and provided supplemental feed, they will start to consume this in greater quantities and rely less on milk production (Sulabo et al., 2010c). It seems that when piglets are weaned older, the benefits of providing supplemental feed on sow performance becomes more apparent. It is possible that if feed intake was greater for young piglets, sow performance may also be improved when piglets are weaned at 21 days of age. However, more research is required to determine the amount of creep feed intake required to positively impact sow performance.

1.2.2 Creep Feeding and Pre-Weaning Piglet Performance

Creep feed form and composition impacts consumption of creep feed by the piglets. The typical composition of creep feed used commercially includes corn, fishmeal, wheat, and a variety of vegetable meals, supplemented with vitamins, minerals, amino acids, and flavorings. However, this composition can vary between companies and regions. Some studies have experimented with changing creep feed formulation such as providing a reduced quality of feed (e.g., supplying piglets with a standard nursery feed or the lactation diet of the sow; Heo et al., 2018), or improving the quality of the feed (e.g., creating a pelleted feed similar in composition to milk replacer; Kim et al., 2001). For example, when provided a feed formulated for sows as a creep feed, piglets had reduced feed intake, average daily gains and weaning weights compared to those given commercial creep feed (Heo et al., 2018). Furthermore, when given pelleted creep feed formulated to match the composition of milk replacer (i.e. whey powder and skim milk as main ingredients instead of corn), piglets had lesser average daily gain, feed intake and took more days to market, than those fed the liquid version (Kim et al., 2001). However, when piglets were provided either a simple (i.e. using plant-based ingredients) or a complex creep feed (i.e. including whey and fishmeal) differences in creep feed intake was only significant for the last 3 days prior to weaning and did not impact weaning body weights at 22 days of age (Collins et al., 2013). Additionally, those provided the simple creep feed had greater average daily gain and feed intake for the first five days after weaning, and this difference was more pronounced when weaned later (i.e. 29 vs. 22 days of age; Collins et al., 2013). The differences in piglet performance when provided a liquid milk replacer instead of a pelleted creep feed is more consistent between studies, with pigs having greater growth rates, feed intake and therefore, weaning body weights when given milk replacer, even when provided pelleted creep feed with a similar formulation as the milk replacer (Kim et al., 2001). Therefore, based on current knowledge, both creep feed form and composition affect feed intake and growth pre-weaning.

Milk replacer is an ideal way to increase energy and nutrient intakes since it is consumed at a younger age and more consistently by piglets (versus pelleted creep feed; Kim et al., 2001). For example, when piglets are given a pelleted creep feed, growth rates were between 2-5% greater than those of piglets given no supplemental feed (Sulabo et al., 2010a; Park et al., 2014; van Oostrum et al., 2016), but growth rates improve by 10-23% when a liquid milk replacer was provided (Wolter et al., 2002; Park et al., 2014). The improvement of average daily gains of piglets fed a milk replacer results in heavier weaning body weights. Weaning body weights tended to increase by 1-2% when provided creep feed (Sulabo et al., 2010a; Muns and Magowan, 2018) and between 3-24% when provided a milk replacer (Azain et al., 1996; Wolter et al., 2002; van Oostrum et al., 2016). Therefore, by providing creep feed or milk replacer during the suckling phase, average daily gain and weaning weight are increased. However, most studies compare either milk replacer or creep feed supplementation to a negative control (no creep feed), which makes it difficult to determine if the high cost of milk replacer is justified by the improvement of growth performance versus conventional creep feed.

1.2.3 Creep Feeding and Post-Weaning Pig Performance

At weaning, piglets are subjected to stress associated with abrupt changes in environment, diet, and pen mates, which contributes to reduced feed intake early after weaning (Colson et al., 2012). This period of reduced energy intake has detrimental effects on the pigs with respect to growth, digestive physiology, and immunology (Engle, 1994; Bruininx et al., 2004). Many researchers have demonstrated an improvement in feed intake following weaning when piglets previously consumed creep feed (Heo et al., 2018; Muns and Magowan, 2018), and some even observed a reduction in feeding latency after weaning (Bruininx et al., 2002). However, these improvements in post-weaning growth are inconsistent as some researchers have not seen any improvement in growth performance after weaning when piglets were provided with creep feed during the suckling period (Delumeau and Meunier-Salaün, 1995; Cabrera et al., 2013).

Since creep feed is in a similar form (pelleted) as nursery diets, it is expected that nursery diets would be consumed more readily when creep feed was previously consumed. However, with the variable feed intake of creep feed among piglets, the extended benefits after weaning are equally variable (Bruininx et al., 2002; Collins et al., 2013). Conversely, milk replacer is consumed in greater quantities, by a greater proportion of piglets, therefore resulting in more consistent improvements in post-weaning growth performance (Vila and Tummaruk, 2016). Despite milk replacer not being in a pelleted form, researchers have observed improvements in feed intake and average daily gain for up to three weeks after weaning when piglets were offered liquid milk replacer during the suckling phase (Dunshea et al., 1999; Park et al., 2014; van Oostrum et al., 2016). The improvements in growth performance of pigs provided a milk replacer during the suckling period are more profound than those of pigs provided a pelleted creep feed (Park et al., 2014). For example, Park at al. (2014) reported that piglets provided milk replacer had greater body weight at the end of the nursery period than those that had been provided a pelleted creep feed, or no creep feed. However, by the grower/finisher phase these differences were no longer apparent. Alternatively, Kim et al. (2019) found that although the average daily gain and feed intake in the grower/finisher phase is not different between pigs that received various creep feeding treatments (i.e. liquid vs. pelleted vs. no creep feed), the improvement in growth throughout the nursery translated to younger pigs reaching market weight (Kim et al., 2001). Therefore, creep feed, and to a greater extent milk replacer, have the potential to improve post-weaning feed intake and average daily gain, the effects of which can be seen until market. Additionally, providing creep feed or milk replacer during the suckling phase resulted in heavier body weight at weaning, that also resulted in fewer days to market (Mahan & Lepine, 1991). However, to see these benefits, piglets must consume the feed and, as studies have shown, consumption is variable.

1.3 NURSERY DIET COMPLEXITY

Creep feed is one strategy for reducing the post-weaning growth-lag by increasing familiarity with pelleted fed, therefore reducing the feeding latency after weaning (Heo et al., 2018). However, nursery diet composition is also important in maintaining growth. Since pigs consume relatively low amounts of nursery feed but have high growth potential, the feed needs to be nutrient-dense to meet energy and nutrient requirements. Additionally, at 21 days of age, the digestive tract of the pig is still underdeveloped (Boudry et al., 2004). Specifically, newly weaned pigs have a relatively high stomach pH and low activity of protein- (e.g. trypsin) and carbohydrate- (e.g., amylase, maltase, sucrase) degrading enzymes that are required to utilize plant-based ingredients (e.g., corn and soybean meal) compared to the mature pig (Whang et al., 2000; Chen et al., 2020). Moreover, some ingredients, such as soybean meal, have antigenic properties that result in inflammation in the gut leading to villus atrophy, further reducing absorptive capacity and increasing the incidence of diarrhea (Engle, 1994; Koo et al., 2020). To combat this, producers provide nursery diets that are nutrient-dense, palatable, and highly digestible (Lee et al., 2019). The aim is to promote feed intake quickly after weaning to maintain pre-weaning average daily gain (Lee et al., 2019).

Nursery diets are typically formulated with whey, blood plasma, and fishmeal in the early phases, which are expensive but can be readily utilized by young pigs (Skinner et al., 2014). These ingredients are much more expensive than the plant alternatives and rely on the availability of animal (by-)products (Skinner et al., 2014). There have been some efforts to feed nursery pigs low-complexity diets (mainly based on corn and soybean meal) to reduce nursery feed costs; however, growth performance is also reduced, at least early in the nursery period (Wolter et al., 2003; Skinner et al., 2014). Typically, when fed a low-complexity nursery diet, pigs experience reduced growth for the duration of feeding this diet (Whang et al., 2000; Skinner et al., 2014; Koo et al., 2020). However, growth can be improved during this time by supplementing with low amounts of milk by-products, fishmeal, insect meal, or fish oil (Huber et al., 2018; Yoo et al., 2018; Koo et al., 2020; Crosbie et al., 2021). Additionally, researchers that provided a low-complexity nursery diet to pigs observed reduced average daily gain for the duration of feeding the diet, followed by a period of accelerated growth during the grower/finisher phase, known as compensatory growth, with no difference in days-to-market or carcass or meat quality at slaughter (Skinner et al., 2014). This indicates that low-complexity diets can be fed in the nursery period and, although growth performance can be reduced while fed this diet, it can be recovered later in the production cycle. The utilization of low-complexity diets can be improved by the addition of certain ingredients, but limited research has been conducted to see if creep feed or milk replacer consumption can improve the utilization of low-complexity nursery diets after weaning.

1.4 GASTROINTESTINAL DEVELOPMENT, MORPHOLOGY, AND PHYSIOLOGY

The digestive system of the neonatal pig undergoes rapid development during the first six weeks of life. From birth and until three weeks of age, the digestive system is adapted to digest and utilize sow's milk (McDonald et al., 2010). Sow's milk is the main source of nutrients during the suckling period and typically limits piglet growth by between 11 and 13 days of age (Strathe et al., 2017), after which, piglets are motivated to find alternative sources of nutrients. As discussed previously in this review, creep feed, and to a greater extent, milk replacer improves pre-weaning growth rates and weaning body weight (Sulabo et al., 2010a; Park et al., 2014). Additionally, creep feed introduces the piglet to consuming ingredients other than milk, initiating maturation of the gastrointestinal tract (Heo et al., 2018).

Typically, for commercially reared pigs, abrupt weaning occurs between 21 and 28 days of age. The adaption to weaning can be broken into two phases: the acute and the adaptive phase (Burrin and Stoll, 2003). The acute phase of weaning occurs directly following weaning (e.g., 5-7 days), average daily gains lower than pre-weaning levels accompanied by villus atrophy typically indicates the animal is in the acute phase, while the adaptive phase can span 14 days thereafter, and is a time where average daily gain and villus height is restored, and surpasses pre-weaning levels (Burrin and Stoll, 2003). Furthermore, the composition of the nursery diet influences the digestive system, as some ingredients (e.g., whey and skim milk) are readily available and do not require mature digestive organs to digest (Pluske et al., 2003), while other ingredients (e.g., soybean meal) can be damaging to the digestive system (Kelly and King, 2009; Lee et al.,

2019) and their inclusion in nursery diets result in greater impairment during the acute phase of weaning. Creep feeding and nursery diet complexity both affect the development of the gastrointestinal tract, and these can be examined by measuring overall size of these digestive organs, histomorphological measurements (e.g., villus height, crypt depth and crypt width), and specific enzyme activities (Hampson and Kidder, 1986; Koo et al., 2020), which are described in detail below.

1.4.1 Visceral Organ Development

When a pig is born, the digestive system is not fully developed (McDonald et al., 2010). There are factors in colostrum and in milk that stimulate rapid growth of the piglet and the digestive organs during the suckling period (e.g., epidermal growth factors and insulin-like growth factor-1; Burrin et al., 1992). As pigs mature, the visceral organs also grow. However, the stress associated with weaning resulted in heavier relative organ weight.

Although visceral organs are essential to utilize dietary nutrients, they are energetically costly to maintain. When visceral organs make up a greater proportion of the pig's body weight, growth (protein deposition) can be limited due to increased maintenance energy requirements (Nyachoti et al., 2000). For example, Jones et al. (2012) found that pigs experiencing the greatest reduction in average daily gain after weaning had heavier visceral organs (e.g., intestines weighing 57.9 vs. 73.3 g/kg body weight for normal vs. pigs experiencing the greatest post-weaning growth lag). Nursery diets typically contain high inclusions of corn, which is less digestible for a young pig than sow's milk. This transition from milk to a less-digestible diet increases the relative weight of the visceral organs (Pluske et al., 2003; Jones et al., 2012). Additionally, the increase in visceral organ mass has also been observed to be greater for pigs fed a high-fiber (less-digestible) cereal-based diet after weaning (Burrin and Stoll, 2003). Therefore, when the pig's diet is changed from a highly digestible diet to a less-digestible one, the relative weights of visceral organs increase. However, limited research has been conducted on the effects on relative organ weight when pigs are provided creep feed or milk replacer prior to weaning, followed by the provision of a low- or a high-complexity diet after weaning.

1.4.2 Gut Morphology

The amount of surface area available for absorption in the gastrointestinal tract is referred to as absorptive capacity. The process of weaning is highly disruptive to gut structure and function. During the acute phase of weaning, villus height is reduced, and crypt depth increases, compared to pre-weaning values, effectively reducing surface area in the small intestine. Crypt depth remains greater after weaning, but villus height is restored to pre-weaning values by 15 days after weaning (Nabuurs et al., 1993; Burrin and Stoll, 2003). However, the duration and severity (e.g., lower villus height, greater crypt depth and lower villus height to crypt depth ratio) of the acute and adaptive phases can be affected by management pre-and post-weaning (Van Beers-Schreurs et al., 1998; Muns and Magowan, 2018). For example, if piglets are offered a less digestible creep feed, such as feed typically given to older pigs, villus height and growth rate are lower prior to weaning, but are less affected by weaning (Heo et al., 2018). Alternatively, when milk replacer is provided after weaning, gut morphology is also maintained (van Oostrum et al., 2016). It seems that the sudden changes in diet quality significantly impacts gut morphology. In the literature, there is not a definitive answer as to whether creep feed will improve intestinal morphology directly following weaning, as some sources suggest less digestible diets (e.g., increased cereal grain inclusion versus whey powder) provided during the suckling period will assist in the maintenance of intestinal structure while others have reported improved intestinal morphology when a high-quality creep feed was consumed (Heo et al., 2018). However, it is possible that by providing creep feed, pigs will become adapted to consuming that diet, and gut architecture can be maintained after weaning. More research is required to determine the effects of creep feed and milk replacer on intestinal morphology and function, specifically how these are maintained following weaning. Creep feed intake is highly variable between and within litters, which is likely why the results regarding gut morphology are variable. If creep feed consumption is more consistent, perhaps more definitive conclusions can be made regarding post-weaning intestinal morphology.

1.4.3.1 Determination of Specific Enzyme Activity

Digestive enzymes that act in the small intestine are categorized into pancreatic and brush border enzymes. Pancreatic enzymes are synthesized in the pancreas and transported to the duodenum via the bile duct (McDonald et al., 2010). The pancreas produces carbohydrate- (i.e. α-amylase), protein- (e.g., trypsin, chymotrypsin, and carboxypeptidases) and lipid- (e.g., lipase) degrading enzymes that yield small oligomers (McDonald et al., 2010). The jejunum has the greatest activity of brush border enzymes, which are present on the villi, typically located on the distal end of the villus. Brush border enzymes are synthesized in enterocytes and then anchored in the apical border of the enterocyte (Hooton et al., 2021). The brush border enzymes break down nutrients even further to produce monomers (e.g., glucose, fructose, galactose, amino acids) so they can be absorbed either through passive diffusion, endocytosis or via transporters (Hooton et al., 2021). The expression and activity of these enzymes change based on age, diet, and location in the gastrointestinal tract, and will be discussed further in this literature review. To determine the differences in activity there are many techniques that can be employed.

Enzymes are made up of proteins and to determine enzyme activity that accounts for the purity of the enzyme, its activity must be reported in terms of total protein, referred to as specific enzyme activity. There are multiple methods that will measure total protein; one of the most common is using the Bradford method. This method is relatively easy to perform and sensitive (Sapan and Lundblad, 2015). This method uses Coomassie Brilliant Blue G-250 that turns from a brown to a bright blue when bound to protein with a maximum absorbance of 595 nm (Bio-Rad; Hercules, CA, USA). This dye preferably binds to basic and aromatic amino acids and can be interfered by detergents and basic buffers (Bio-Rad; Hercules, CA, USA). This method requires comparison to a standard curve using a protein standard of known protein concentration; however, if the standard is different in composition than the protein of interest this can cause bias in the results of the assay (Shen, 2019). Another common method for protein determination is the Lowry method. This assay measures protein concentration by measuring cuprous complexes, which are formed by the interaction of copper and nitrogen that are present in peptides (Shen, 2019). This method has a similar range as the Bradford method, but it requires multiple steps to perform and many common substances interfere with this method (Sapan and Lundblad, 2015; Shen, 2019). Similar to the Bradford method, the Lowry method also uses a protein standard to determine protein concentration in the samples, if the standard and sample proteins are not similar in composition this can impact the results of this method (Shen, 2019).

The determination of activity for enzymes can be done in multiple ways using a spectrophotometer. Special considerations when designing a method for enzyme activity determination include pH, temperature, and time (Bisswanger, 2014). The time required for the enzyme reaction must be determined by performing a time-course study, to see how long it takes for the enzymes in the sample at a specific pH and temperature to reach the linear phase and plateau of product formation (Bisswanger, 2014). To compare enzyme activities in samples, an incubation time in the linear phase must be selected (Bisswanger, 2014).

For brush border enzymes such as maltase, sucrase and lactase, enzyme activity can be determined by the production of glucose. Maltose is made up of two glucose monomers, sucrose is made up of one glucose and one fructose, and lactose is composed of one glucose and one galactose (McDonald et al., 2010). The monomer sugars are released when the respective enzymes break the glycosidic bond and, when a known amount of substrate is added to the reaction, greater production of glucose indicates greater specific enzyme activity. In one method, glucose oxidase is added to the sample and reacts with the free glucose, producing hydrogen peroxidase (Sigma-Aldrich, Oakville, ON, CAN). In the presence of horseradish peroxidase, the hydrogen peroxide generates a colour reaction that can be measured using a spectrophotometer (Sigma-Aldrich, Oakville, ON, CAN). The glucose concentration can then be calculated by comparing absorbance of the sample to a standard.

Pancreatic enzyme activity can be measured in the pancreas, pancreatic juice, and intestinal contents (Gorrill and Thomas, 1967). These enzymes (e.g., trypsin and chymotrypsin) are synthesized in the pancreas in the inactive forms and are transported to the duodenum via the pancreatic duct and bile duct (Gorrill and Thomas, 1967). Enterokinase, which is present as a brush border enzyme in the duodenum, activates trypsinogen to trypsin, and then trypsin activates multiple pancreatic enzymes including chymotrypsinogen (Kunitz, 1939). Intestinal contents can pose some challenges for the determination of enzyme activity, as there can be interfering substances (i.e. bile salts) present that would need to be removed prior to analysis (Gorrill and Thomas, 1967). Additionally, pancreatic enzymes are released into the duodenum in response to available substrates; therefore, the activity of these enzymes can be altered depending on the fasting interval of the animal (Chandra and Liddle, 2009). If measuring enzyme activity from a pancreas or mucosal sample, the tissue must first be homogenized, whereas pancreatic juices do not require homogenization. However, both tissues and pancreatic juices require activation. Once samples are homogenized and activated, the appropriate substrates can be added (e.g., toluene arginine methyl ester for trypsin and benzoyl tyrosine ethyl ester for chymotrypsin; Gorrill and Thomas, 1967). After incubation, enzyme activity can be measured using a spectrophotometer, as above (Gorrill and Thomas, 1967). After enzyme activity has been determined (either brush border or pancreatic), specific activity can be calculated by dividing the enzyme units by the grams or milligrams of total protein in the sample or sample homogenate.

1.4.3.2 The Effect of Age and Diet on Specific Enzyme Activities

A major component of the mucosa of the small intestine includes mucus and digestive enzymes (McDonald et al., 2010). Young pigs' digestive systems are specialized to digest milk, meaning that from birth, the enzyme lactase is produced in large amounts to break up the main disaccharide in milk, lactose (McDonald et al., 2010). Lactase is produced in large amounts until approximately three-to-seven weeks of age, at which point its production declines and is replaced by other enzymes required for digestion of various plant and animal ingredients (McDonald et al., 2010). For example, the production of maltase and sucrase, two other disaccharidases, start to increase at four weeks of age and reach a constant level by eight weeks of age (McDonald et al., 2010). Maltase and sucrase are essential for carbohydrate digestion, and since these levels are low for the first 4-8 weeks of a pig's life, the capacity to breakdown and absorb carbohydrates is limited compared to a mature pig (McDonald et al., 2010). However, weaning disrupts the maturation of the gastrointestinal tract. The acute phase of weaning results in a temporary decline in enzyme activity, followed by an increase past pre-weaning levels in activity during the adaptive phase (Boudry et al., 2004). Lactase is the exception, as it steadily declines after weaning.

At weaning (3-4 weeks of age), pigs transition from consuming sows' milk, to consuming commercial diets largely made up of cereal grains and legumes (e.g., corn, wheat, and soybean meal). The low levels of carbohydrate-degrading enzymes during the nursery phase contribute to the post-weaning growth lag (de Passillé et al., 1989). Creep feed is formulated to be highly digestible by a young pig by utilizing ingredients such as whey powder and skim milk, but also includes corn as a major component (Sulabo et al., 2010c; Yan et al., 2011; Heo et al., 2018). The use of cereal grains in creep feed is theorized to expedite the maturation of the gastrointestinal tract by increasing the activity of digestive enzymes specific for carbohydrates. At weaning, pigs that were provided creep feed had increased relative enzyme activities (i.e. trypsin and chymotrypsin; Friend et al., 1970). Conversely, the specific activities of these enzymes are not always elevated in pigs that consumed creep feed prior to weaning (e.g., Cabrera et al., 2013). However, it seems that the amount of creep feed that was consumed prior to weaning is critical in eliciting an increase in enzyme production (Friend et al., 1970; Engle, 1994). For example, it is suggested that a piglet would need to consume between 400 and 600 g of creep feed throughout the suckling period to see improvements in gastrointestinal maturation after weaning (English et al., 1980; cited by Pluske, 2016). This amount is proposed for commercial creep feeds, and it has not been determined how this value would change depending on creep feed composition (e.g., if corn is not included or included in lower or higher amounts). Furthermore, research suggests that growth rate impacts gastrointestinal maturation, including the production of maltase (Friend et al., 1970; de Passillé et al., 1989). Although both creep feed and nursery diet complexity can individually improve nutrient utilization after weaning, research in this area is limited, and more research is required to determine if the interaction between creep feed and nursery diet complexity can improve enzyme activities after weaning to help alleviate the post-weaning growth lag.

1.4.5 Immune Function

Suckling piglets receive passive immunity through the consumption of maternal immunoglobulins present in colostrum (Farmer et al., 2020). At weaning, serum concentrations of IgG is reduced by ½ to ¼ of pre-weaning levels. Moreover, the piglet's adaptive immune system is immature, leaving the piglet with a compromised ability to mount immune responses (Piñeiro et al., 2019; Farmer et al., 2020). The period when the piglet has low levels of maternally-derived immunity and prior to having a comprehensive adaptive immune system is when weaning typically occurs. Moreover, weaning is a stressful event that involves mixing and transport of pigs, a new environment, and changes in diet composition and form. In-feed antibiotics have traditionally been included in nursery diets to mitigate the incidence of enteric disease post-weaning; these have recently been banned due to concerns regarding antibiotic resistance (PHAC, 2021). Additionally, following weaning, pigs tend to have reduced feed intake causing a thinning of the mucus layer in the intestine (Toth et al., 2015). This mucous layer acts as the first line of defense in the intestine that prevents pathogenic bacteria from attaching to the surface; therefore, with the degradation of this defensive layer, enteric bacteria are more likely to attach and proliferate (Toth et al., 2015). Moreover, the risk of bacterial invasion is worsened since weanling piglets have higher gastric pH, which is favourable for bacteria and less so for enzymatic activity, further increasing intestinal dysfunction (e.g., villus atrophy, crypt deepening, malabsorptive diarrhea) after weaning (Li et al., 2019).

Inflammation in the gastrointestinal tract is normal during development; however, prolonged or excessive inflammation can cause damage to the surrounding tissues. Inflammation can be exacerbated at weaning when soybean meal is included in the diet due to antigenic compounds (Engle, 1994; Koo et al., 2017). These antigenic compounds combined with the underdeveloped immune system of the weanling pig leads to long-term intestinal damage (McDonald et al., 2010). Atrophy of the villi and deepening of intestinal crypts reduce surface area and therefore, absorptive capacity is compromised, reducing feed efficiency (McDonald et al., 2010). To mitigate the degree of inflammation in the gastrointestinal tract, producers feed highly digestible diets during the nursery phase consisting of high inclusions of milk- and animal-based protein sources (McDonald et al., 2010). This means that the cost of the nursery diet is much higher than diets formulated with plant-based protein sources (e.g., spray-dried plasma is 10× more expensive than soybean meal). Although some researchers reported no change in lifetime growth performance, there are differences in immune response of pigs provided a low-complexity diet. For example, Skinner et al. (2014) noted an increase in mortality from 10% to 27% in the nursery and grower/finisher phase when a low-complexity diet without in-feed antibiotics was fed, versus a high-complexity diet. Additionally, Koo et al. (2020) found that pigs fed a low-complexity diet (with greater inclusions of soybean meal) had increased plasma IL-6 and IL-10. Therefore, nursery pigs provided a low-complexity diet seem to exhibit compensatory growth during the grower/finisher phase, but during the nursery phase they have impaired immune system activation and increased intestinal permeability due to low feed intake and sensitization to antigenic compounds found in soybean meal.

1.5 CONCLUSIONS

In summary, genetic selection has resulted in higher growth rates and larger litter sizes in swine (Mabry, 2015). This means that sows, and to a greater extent, first parity sows, cannot meet the nutrient and energy requirements for milk production without mobilizing body stores (Strathe et al., 2017). Moreover, milk yield begins to limit piglet growth prior to weaning. Creep feed can be provided to suckling piglets to supplement milk intake resulting in improved growth rates and body weight at weaning. Furthermore, the familiarity with a pelleted diet could improve feed intake after weaning (Bruininx et al., 2002; Pluske et al., 2003). However, creep feed intake is typically low and highly variable among piglets, therefore limiting the apparent benefits of providing creep feed. Weaning is a time of stress resulting in prolonged fasting and immunological challenges, damage to the gastrointestinal tract, and reduced nutrient utilization for net protein gain, all of which contribute to the post-weaning growth lag (Deprez et al., 1987; Van Beers-Schreurs et al., 1998; Colson et al., 2012). Creep feed can have similar ingredient composition to nursery diets, allowing the pigs' gastrointestinal tract time to adapt to novel, non-milk-based ingredients. Milk replacer is an alternative form of creep feed with a higher, more consistent consumption among piglets, further improving growth rates prior to weaning (Kim et al., 2001; Wolter et al., 2002). However, milk replacer, may not assist in preparing the piglet for the dietary changes associated with weaning since the ingredients used mimic those of sow's milk. Weanling pigs provided a nursery diet formulated mainly with corn and soybean meal (i.e. low-complexity diets) had reduced growth performance (e.g., average daily gain, average daily feed intake and gain to feed), but these effects are typically only present during the nursery period (Skinner et al., 2014; Koo et al., 2020). The interaction between pre-weaning feeding regimen and how it affects the utilization of low-quality nursery diet is not well understood. It is important to determine the optimal feeding strategy with respect to cost and pig performance both pre- and post-weaning to maximize producer profitability. Furthermore, the interaction between pre- and post-weaning feeding strategies has not been studied with regards to immune system activation, gut morphology, and mucosal specific enzyme activity. Based on current knowledge, it is expected that pre-weaning supplemental feed can familiarize piglets to consuming diets other than milk, which can be beneficial to maintaining growth and intestinal morphology following weaning.

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CHAPTER 2: RESEARCH RATIONALE AND OBJECTIVES

During weaning, pigs are faced with social, environmental, and dietary stress in addition to depletion of maternal antibodies, and the underdeveloped immune and digestive systems (Bruininx et al., 2004; Colson et al., 2012; Jones et al., 2012). These result in reduced feed intake and growth, known as the post-weaning growth lag (Bruininx et al., 2004; Collins et al., 2013). Highly digestible nursery diets with high inclusion levels of animal-derived protein sources (e.g., blood plasma, whey, and fishmeal; high-complexity) have typically been used to minimize this growth lag (Whang et al., 2000; Skinner et al., 2014). However, high-complexity nursery diets contribute to high cost of feed after weaning and by using plant-based protein sources (e.g., soybean meal) to generate low-complexity nursery diets can reduce feed costs.

Creep feed can be provided to piglets during the sucking period to improve piglet growth performance prior to weaning and improve growth and feed intake after weaning (Park et al., 2014). Milk replacers have higher feed intake than creep feeds but are expensive to provide and are not in the same form as nursery diets (i.e. liquid versus pelleted; Park et al., 2014). A pelleted milk replacer is more cost effective to supply and is in the same form as the pelleted diets, but like the milk replacer, is formulated with different ingredients from those of a nursery diet, limiting habituation to post-weaning diet ingredients. It is hypothesized that the provision of creep feed will assist in the development of the nursing pig's digestive system, enabling better utilization of low-complexity nursery diets after weaning.

The specific objectives of this thesis were to determine the effects of creep feed composition and form and nursery diet complexity on:

1. Piglet and sow body weight change, feed intake, feed efficiency, nutrient digestibility and visceral organ development during the suckling and nursery periods, and determine feeding costs when providing various creep feed and nursery diet treatments
2. Pig gut development (e.g., gut morphology and specific enzyme activities of maltase, sucrase and lactase), and immune response (e.g., OVA-specific IgG) after weaning

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CHAPTER 3: THE EFFECT OF CREEP FEED COMPOSITION AND FORM ON PRE- AND POST-WEANING GROWTH PERFORMANCE OF PIGS AND THE UTILIZATION OF LOW-COMPLEXITY NURSERY DIETS

3.1 Abstract

Fifty-six litters from first-parity sows standardized to 12 piglets were used to determine the effects of creep feed composition and form on pre- and post-weaning pig growth performance and the utilization of low-complexity nursery diets. At five days of age, litters (initial BW 2.31±0.61 kg) were assigned to one of four creep feeding regimens (n=14): [1] commercial creep feed (**COM**), [2] liquid milk replacer (**LMR**), [3] pelleted milk replacer (**PMR**), or [4] no creep feed (**NO**); creep feeds contained 1.0 % brilliant blue as a fecal marker. Individual piglet BW and fecal swabs were collected every 3±1 days during the creep-feeding period. The latter was to identify piglets that regularly consumed creep feed via the visual appearance of blue dye in the feces. At weaning (21±2.1 days of age), six pigs per litter with median BW that consumed creep feed were placed on either a **HIGH-** (contained highly digestible animal proteins) or **LOW-** (contained corn and soybean meal as the main protein sources) complexity nursery diet (n=7) in a three-phase feeding program over 38 days. On day 7 after weaning, two pigs per pen were slaughtered to collect organ weights and digesta. During the creep feeding period, The LMR disappeared at a greater rate (average 37.7 g/pig/d; DM-basis) than COM and PMR (10.6 and 10.3 ± 1.5 g/pig/d, respectively; $P < 0.001$). Litters that received LMR had the greatest proportion of pigs with blue fecal swabs throughout the creep feeding period (85.0 vs. 54.9 and 63.0 ± 0.4% for COM and PMR, respectively; $P < 0.05$) and LMR piglets had greater BW at weaning versus all other treatments (6.32 vs. 6.02, 5.92, and 5.67 ± 0.14 kg, for LMR, COM, NO, and PMR, respectively; $P < 0.001$). Overall, pigs given LOW (versus HIGH) diets in the nursery period had reduced ADG (25.1 vs. 27.7 ± 0.4 g/kg BW; $P < 0.001$), G:F (0.75 vs. 0.81 ± 0.02; $P < 0.001$), and exit BW (21.2 vs. 24.4

± 0.6 kg; $P < 0.001$); no carryover effects of creep feeding program were observed. Creep feed regimen had limited effects on nutrient digestibility of nursery diets, but the apparent ileal digestibility of OM tended to be less at 28 days of age for pigs that received the LOW nursery diet (64.2 vs. 68.8 ± 2.5%; $P = 0.076$). Providing supplemental nutrition during the suckling period via LMR improved piglet BW at weaning, which did not correspond to improved post-weaning growth performance, regardless of nursery diet complexity.

3.2 Introduction

Providing supplemental feed to piglets (i.e. creep feed) during the lactation period is one strategy to increase energy and nutrient intakes of piglets, maximize piglet BW at weaning, and potentially, reduce nutrient demands for the lactating sow (Miller et al., 2012; Novotni-Dankó et al., 2015). Creep feed consumption is highly variable between and within litters (Pajor et al., 1991; Bruininx et al., 2002), but piglets that consume creep feed have greater ADG during the lactation period and BW at weaning (Wolter et al., 2002; Sulabo et al., 2010; Park et al., 2014). Moreover, exposure to pelleted creep feed habituates piglets to consuming a pelleted diet, which can improve ADFI and ADG after weaning (Pluske et al., 2007; Muns and Magowan, 2018). Alternatively, providing supplemental nutrition as liquid milk replacers can reduce variability in creep feed intake among piglets and improve ADG, as well as BW at weaning versus those provided with pelleted creep feed or no creep feed (Kim et al., 2001; Bruininx et al., 2002; van Oostrum et al., 2016). However, milk replacers do not introduce piglets to plant-based protein sources and do not familiarize piglets with solid (pelleted) diets. Moreover, liquid milk replacers require specialized feeding equipment and/or increased manual labor versus providing pelleted creep feeds (e.g., for frequently dispensing milk replacer and maintaining feeder cleanliness).

Typically, nursery diets are formulated with highly digestible and expensive protein sources (e.g., whey powder, fishmeal, blood meal) to optimize nutrient absorption and minimize the post-weaning growth lag (Koo et al., 2020). Previous research has demonstrated however, that pigs fed nursery diets with lower quality protein sources (e.g., soybean meal) can accelerate growth after an initial reduction in ADG and achieve the same BW as those fed high quality protein sources immediately after weaning (i.e. compensatory growth; Skinner et al., 2014; Huber et al., 2018). However, it is not known whether piglet nutrition during the lactation period can influence the utilization of low-complexity nursery diets. Therefore, the aim of the current study was to determine the effects of creep feed composition and form on pre- and post-weaning pig growth performance and the utilization of low-complexity nursery diets.

3.3 Materials and Methods

3.3.1 Animals and Housing

The experimental protocol was approved by the University of Guelph Animal Care Committee and followed Canadian Council on Animal Care guidelines (CCAC, 2009; AUP #4044). The study was conducted at the University of Guelph Arkell Swine Research Station (Guelph, ON, Canada).

Six hundred seventy-two piglets born to 56 Landrace × Yorkshire first-parity sows (initial BW 190.3 ± 1.33 kg) mated with Duroc boars over seven breeding batches (blocks) were used for the study. Litters were standardized to 12 piglets within 48 hours of parturition. At 4 days of age, all pigs were tail-docked, given an intramuscular injection of 200 mg of iron dextran, an intramuscular injection of 0.5 mg of meloxicam (Metacam for swine, Boehringer-Ingelheim, Burlington, ON, Canada) and males were castrated. During the suckling period, piglets were housed with the dam in conventional farrowing crates (183 × 241cm) with a heating pad (35°C) in the creep area. All piglets had unlimited access to water via nipple drinkers. Sows were fed 2 kg of standard lactation diet (CP 17%, NE 2519 Kcal/kg; Appendix A) from day 110 of gestation until farrowing. After parturition, feed was provided in a stepwise manner for 4 days when *ad libitum* feed allowance was reached. Sow feed intake was recorded weekly. The interval between weaning and breeding was recorded for each sow and sows were only bred when signs of heat were present.

At weaning, piglets were vaccinated with 1 mL of porcine circovirus type 2 vaccine (Ingelvac Circo Flex, Boehringer Ingelheim, Burlington, ON, Canada), 2 mL of a combination *Mycoplasma hyopneumoniae-Glaesserella parasuis* vaccine (Suuvazyn MH/HPS, Zoetis Canada Inc, Kirkland QC, Canada) and 2 mL of *erysipelothrix rhuiopathiae* vaccine (ER Bac Plus, Zoetis Canada Inc, Kirkland, QC, Canada) with boosters given of Suvazyn MH/HPS and ER Bac Plus 3 weeks later. Six piglets per litter (3 castrated males and 3 females) of median BW were selected to continue the study and were weaned into a nursery pen (112 × 147 cm; one pen per litter) with plastic-coated, expanded metal floors in an environmentally controlled nursery room. Room temperature was 27°C during the first week after weaning and reduced by 1°C each week thereafter. Each pen contained a stainless-steel feeder with four head spaces, a nipple drinker, and a toy for enrichment.

3.3.2 Dietary Treatments and Feeding

Starting at 5 ± 0.3 days of age (initial BW 2.38 ± 0.02 kg) and continuing until weaning at 21 ± 2.1 days of age, litters were provided with one of four creep treatments according to a randomized block design: [1] commercial creep feed (**COM**), [2] liquid milk replacer (**LMR**), [3] pelleted milk replacer (**PMR**), or [4] no creep feed (**NO**; n=14). The COM contained corn, fishmeal, and soybean meal (Floradale Feedmill Ltd., Floradale, ON, Canada), while the LMR (powder) and PMR were formulated with matched levels of net energy (2903 kcal/kg), crude protein (22 %), crude fat (10 %), and standardized ileal digestible (**SID**) Lys (1.61 %), milk protein (16%) and starch (8%) supplied by milk products (Grober Nutrition, Cambridge, ON, Canada; Table 3.1). The PMR contained 12 % corn to facilitate pelleting. All creep feeds and milk replacer powder were mixed with 1 % (wt/wt) brilliant blue dye prior to feeding for visual identification of individual piglets that consumed creep feed via appearance in the feces (Agyekum et al., 2018). The blue dye was included with the milk replacer powder and was coated onto the pelleted diets using canola oil (i.e. 5 mL of oil per 300 g of pellets). The creep feeds and milk replacer were provided in MS Schippers MS Click Feeder Mini (1.5 L capacity; MS Schipper, Lacombe County, AB, Canada). Feeders were placed in the creep area at the front of the crate, adjacent to the heating pad. Litters that received the PMR or COM creep treatments were given 300 g of feed at 0930 hr once daily. Litters that received the LMR creep treatment were given fresh milk replacer twice daily at 0930 hr and 1430 hr. For the first 7 days of creep treatment, litters provided with LMR were fed daily portions of 1.5 L (250 g of milk replacer powder per liter of warm water), 2 L for the subsequent 4 days, and 3 L thereafter until weaning. Creep feed disappearance was determined daily on a DM basis.

At weaning, six pigs from each litter that were classified as ‘eaters’ (i.e. produced fecal swabs positive for blue dye at least on days 17 and 21 of age) were selected and placed in a nursery pen (1 pen per litter; pen was the experimental unit). Nursery pens were randomly assigned either a **HIGH-** (contained highly digestible animal protein sources) or **LOW-** (contained corn and soybean meal as the main protein sources; Table 3.2) complexity nursery diet according to a 4×2 factorial arrangement (i.e. with creep treatment and nursery diet as the two factors; n=7). Nursery diets were fed in a three-phase feeding program with phases I, II, and III fed for 7, 14, and 17 days, respectively. Diets were formulated to meet estimated nutrient requirements for nursery pigs within each phase (NRC, 2012). The phase I diets contained 0.3% titanium dioxide as an indigestible marker. Phase III also contained 0.2% titanium dioxide at the expense of corn during the final 10 days of the study.

3.3.3 Experimental Procedures

Backfat and loin depth of sows were measured at the P2 position (6.5 cm from the midline over the last rib) on day 110 of gestation and at weaning using a portable ultrasound machine with a 140 mm linear probe (Agroscan L, ECM Noveko International Inc. Angoulême, France). Individual sow BW was determined within 24 hours after parturition and again at weaning.

Individual piglets were weighed upon initiating the creep feed treatments and were weighed and fecal swabbed on 9, 13, 17 and 21 (weaning) days of age to classify individual piglets as creep feed ‘eaters’ or ‘non-eaters’ via the presence of blue dye in the feces. In the nursery period, individual pigs were weighed and per pen feed disappearance was determined weekly to calculate ADG, ADFI, and G:F for each phase.

Cameras (Canon Vixia RF800; Brampton, ON, Canada) were installed above each nursery pen and recorded continuously for 48 hours after weaning. Individual pigs within each pen were uniquely identified with animal marker that was visible in the video recording. The latency to consume the nursery diet was determined for each individual pig; a feeding event was noted when the pig placed its head in the feeder for three or more seconds or was observed chewing (adapted from Pajor et al., 1991).

At weaning (21 days of age), one-week post-weaning (28 days of age), and at the end of the nursery period (59 days of age), 2 pigs per litter were randomly selected (1 castrated male and 1 female; 14 pigs per treatment) and were euthanized with an intra-cardiac injection of 3 mL of Euthasol (Virbac, TX; days 21 and 28) or by electrical stunning followed by exsanguination (day 59). Immediately thereafter, the entire gastrointestinal tract was excised, and gut and organ weights were measured. Ileal digesta from the last meter of the small intestine was collected on days 28 and 59. Fresh fecal samples were also collected from pens between days 57 and 59 and from the descending colon during dissection. Both ileal digesta and fecal samples were pooled by pen. Ileal and fecal samples were stored at -20°C until freeze drying and then were ground and stored at 4°C until further analysis.

3.3.4 Nutrient Analysis

Subsamples of each creep feed and nursery diet were collected weekly and combined within phase (for nursery diets) and were finely ground using a coffee grinder (Custom Grind Coffee Grinder, Hamilton Beach Brands Canada Inc., Belleville, ON, Canada). Ground samples were analyzed for DM (AOAC 2005; method 930.15), CP (AOAC 2005; method 968.06), calcium, phosphorus, potassium, and magnesium using inductively coupled plasma mass spectrophotometry (AOAC, 2005; method 985.01; creep diets only) and sodium using inductively coupled plasma-optical emission spectrometry (AOAC, 2005; method 2011.14; creep diets only; Agrifood Laboratories, Guelph, ON, Canada).

Freeze-dried ileal and fecal samples and nursery diet composite samples (phases I and III only) underwent analyses for DM (AOAC, 2005; method 930.15), ash (AOAC, 2005; method 942.05) and nitrogen (via combustion; LECO-FP 828 analyzer, LECO Instruments Ltd, Mississauga, ON, Canada). Titanium content was determined according to Myers et al. (2004) with minor adaptations (digestion for 24-h at 120 °C in 10 mL tubes and addition of H_2O_2 after precipitate settled in 100 mL volumetric flasks) and absorbance of standards and samples were measured by spectrophotometry at 408 nm (Epoch 2, BioTek Instruments Inc.Winooski, VT). The gross energy (**GE**) of nursery diet composite samples and fecal samples was determined via a bomb calorimeter (IKA Calorimeter System C 5000; IKA Works Inc., Wilmington, NC). For all analyses, diet samples were analyzed in quadruplicate and ileal and fecal samples analyzed in triplicate.

3.3.5 Calculations and Statistical Analysis

Apparent ileal digestibility (**AID**) of organic matter (**OM**) and CP and apparent total tract digestibility (**ATTD**) of OM and GE were calculated according to Fuller et al. (2012). Feed costs for the creep feed and nursery treatments were calculated per pig and per kilogram of pig BW at weaning and exit from the nursery, respectively, using current commodity prices. The statistical analysis for lactating sow performance (changes in body weight, back fat, loin depth and weaning-to-estrus interval) and suckling and nursery pig growth performance (ADG, ADFI, G:F, relative organ weights) and feed costs were conducted using the GLIMMIX procedure of SAS (University Edition; SAS Inst. Inc., Cary, NC) with either creep feed treatment as the main effect (prior to weaning) or creep feed treatment, nursery diet, and the interaction between creep feed treatment and nursery diet as the main effects (after weaning). The interaction between creep feed treatment and nursery diet was generally not significant, therefore, only the main effects of creep feed treatment and nursery diet are presented for all nursery outcomes, except for pig BW and overall ADG. Sow and litter (prior to weaning) or pen (after weaning) were the experimental units. Block was considered a random effect and initial BW (i.e. at the start of creep feeding) was used as a covariate, when appropriate. The proportion of piglets that had blue fecal swabs on each sampling day was analyzed as a binary distribution to determine the odds of each piglet having a blue fecal swab. In all analyses, the degrees of freedom were calculated with Kenward-Roger's adjustment for repeated measures and outliers were detected using the univariate procedure. Model residuals were assessed using scatter and box plot of studentized residuals for homogeneity of variance, Q-Q plot and Shapiro-Wilk test for normal distribution. Mean comparisons were conducted using Tukey-Kramer test to separate means. Probability (***P)***-values of less than 0.05 were considered significant, and $0.05 \leq P \leq 0.10$ were considered tendencies.

3.4 Results

The analyzed and calculated nutrient contents for the creep feed treatments were comparable (Table 3.1). The analyzed and calculated nutrient contents were also generally comparable for the nursery diets (Table 3.2). The exception was for the phase I diets where the analyzed Ca content was 20% lower versus calculated for the LOW diet and the analyzed P contents were 15 and 24 % lower versus calculated for the LOW and HIGH diets, respectively. During the suckling period, 28, 14, 8, and 7% of individual piglets that received the LMR, PMR, COM, and NO creep feed treatments, respectively, were treated for diarrhea, though there was no difference in mortality (~ 2 %) during the creep feeding period among treatments (n=14). In addition, neither creep nor nursery treatment affected mortality in the nursery period (~ 1 %; n=7).

3.4.1 Lactation Performance

Initial body weight for sows among all creep treatments were not different (Table 3.3). Creep feed treatment did not influence changes in sow BW, backfat depth, loin depth, or ADFI during the lactation period or the wean-to-estrus interval. Initial body weight of piglets and litter size were not different among creep feed treatments. The ADG over the creep feed treatment period was greater for piglets that received the COM and LMR treatments versus those that received the PMR treatment ($P < 0.05$); intermediate values were observed for piglets that received the NO treatment. The average daily creep feed disappearance (DM-basis) was greater for piglets that received the LMR versus the COM and PMR treatments ($P < 0.05$), which were not different. Piglets that received the LMR had greater BW at weaning than all other creep treatment groups ($P < 0.05$), piglets that received the COM had greater BW at weaning than those that received the PMR ($P < 0.05$), and piglets that received the NO treatment had intermediate BW at weaning versus the COM and PMR groups. The estimated cost of creep feed per pig and cost of creep feed per kilogram of BW at weaning were greater for piglets that received LMR versus those that received COM and PMR ($P < 0.001$), which were not different.

The average daily creep feed disappearance between each fecal swabbing period and the occurrence of piglets with blue feces (‘eaters’) were greater for piglets that received the LMR treatment versus other groups ($P < 0.05$) but were generally not different between piglets that received the COM and PMR treatments (Table 3.4). Only on 13 days of age did piglets that received the PMR treatment have a greater occurrence of blue feces than those that received the COM treatment ($P < 0.05$) and between days 14 and 15, piglets that received the PMR had lower average daily creep feed disappearance than those that received the COM treatment ($P < 0.05$).

3.4.2 Nursery Growth Performance

After weaning, neither creep feed treatment nor nursery diet complexity influenced the latency to consuming the first meal or apparent daily feed intake during the first 48 hours (Table 3.5). Initial BW upon entering the nursery (21 days of age) was influenced by the interaction of creep and nursery feed treatments (*P* < 0.001) and the main effect of creep treatment (*P* < 0.001; Figure 3.1; Panel A). Within the HIGH nursery diet treatment, pigs that received PMR during the creep feeding period had lower BW on day 21 versus pigs that received all other creep treatments (*P* < 0.05) and within the LOW nursery diet treatment, pigs that received LMR during the creep feeding period had greater BW on day 21 versus pigs that received all other creep treatments (*P* < 0.05). On day 28 (end of phase I), pig BW was influenced by the interaction of creep and nursery feed treatments (*P* < 0.05) and the main effects of creep (tendency; *P* = 0.064) and nursery feed treatments (*P* < 0.001; Figure 3.1; Panel B). Within the HIGH nursery diet treatment, pigs that received PMR during the creep feeding period had lower BW versus pigs fed COM (*P* < 0.05), while the BW of pigs that received LMR and NO were intermediate. Within the LOW nursery diet treatment, there were no differences in BW among creep feed treatments. On day 42 (end of phase II), pig BW was influenced by the interaction of creep and nursery feed treatments (*P* < 0.05) and the main effects of creep (tendency; *P* = 0.067) and nursery feed treatments (*P* < 0.001; Figure 3.1; Panel C). Within the HIGH nursery diet treatment, pigs that received PMR during the creep feeding period had lower BW versus pigs fed COM and LMR (*P* < 0.05), while the BW of pigs that received NO were intermediate. Within the LOW nursery diet treatment, there were no differences in BW among creep feed treatments. On day 59 (end of phase III), pig BW tended to be influenced by the interaction of creep and nursery feed treatments (*P* = 0.086) and the main effects of creep (*P* = 0.065) and nursery feed treatment (*P* < 0.001) but for either nursery diet treatment there were no differences in BW among pigs fed the various creep feed treatments (Figure 3.1; Panel D). On days 28, 42, and 59, BW were less for pigs that received the LOW versus the HIGH nursery diet (*P* < 0.001; Table 3.6).

The per-phase ADG, ADFI, and G:F were not influenced by the interaction of creep and nursery treatments, therefore, only the main effects are presented. In phase I, ADG and G:F were not influenced by the main effect of creep feed treatment, but were less for pigs fed LOW versus HIGH complexity nursery diets ($P < 0.05$; Table 3.6). In phase I, ADFI was greater for pigs that received LMR versus those that received NO during the suckling period ($P < 0.05$), while intermediate values were observed for pigs that received the COM and PMR. In phase I, ADFI was less for pigs fed LOW versus HIGH complexity diets ($P < 0.001$). In phase II, ADG tended to be greater for pigs that received LMR versus those that received NO during the suckling period ($P = 0.098$), while intermediate values were observed for pigs that received the COM and PMR. In phase II, ADFI tended to be greater for pigs that received PMR and NO versus those that received COM during the suckling period ($P = 0.079$), while pigs that received LMR had intermediate ADFI. The G:F in phase II tended to be influenced by creep feed treatment ($P = 0.071$). In phase II, ADG, ADFI, and G:F, were less for pigs that received LOW versus HIGH nursery diets. In phase III, ADFI tended to be greater for pigs that received COM versus LMR in the suckling period ($P = 0.058$), while intermediate values were observed for PMR and NO; ADG and G:F were not influenced by creep feed treatment and ADG, ADFI, and G:F were not influenced by nursery treatment.

Overall (between 21 and 59 days of age), ADG was influenced by the interaction between creep and nursery treatments ($P < 0.05$) where pigs that received LMR or PMR during the creep feeding period and the HIGH nursery diet had greater ADG than all pigs fed the LOW nursery diet, regardless of creep feeding treatment ($P < 0.05$), while pigs fed COM-HIGH, NO-HIGH, COM-LOW, PMR-LOW, and NO-LOW were intermediate (Figure 3.2). Creep treatment provided during the suckling period did not influence overall ADFI and G:F in the nursery; ADG and G:F were less for pigs that received LOW versus HIGH nursery diets ($P < 0.001$), and ADFI was not affected by nursery diet treatment (Table 3.6). Feed cost per pig during the nursery period was not influenced by creep feeding treatment during the suckling period but was less for pigs that received the LOW versus HIGH nursery diet ($P < 0.001$). Cumulative feed cost (i.e. during the creep feeding and nursery periods) per pig and per kilogram of nursery exit BW were greater for pigs that received LMR versus all other creep feeds ($P < 0.05$) and less for pigs that received the LOW versus HIGH nursery diet ($P < 0.001$).

3.4.3 Relative Organ Weights After Weaning

On day 21, live BW and relative weights of the gastrointestinal tract (**GIT**) segments and liver were not affected by creep feed treatment (Table 3.7). On days 28 and 59, live BW was influenced by the interaction of creep feed and nursery treatments, but the interaction was not significant for any other outcome (data not shown). On day 28, live BW was less for pigs that received LOW versus HIGH nursery treatment ($P < 0.05$) but relative GIT segments were not affected by creep feed or nursery treatment, and relative liver weight tended to be influenced by creep feed treatment such that PMR had heavier relative liver weight than NO ($P = 0.089$). On day 59, live BW was influenced by creep feed treatment ($P < 0.05$) and was less for pigs that received LOW versus HIGH nursery diets ($P < 0.001$). The relative full gut weight (g/kg of BW) was greater and small intestine tended to be greater for pigs that received PMR versus those that received LMR during the suckling period ($P < 0.05$ and $P = 0.051$, respectively), while intermediate values were observed for NO and COM. Relative full gut and small intestine weights were also greater for pigs that received LOW versus HIGH nursery treatment ($P < 0.01$). Relative empty stomach weight was greater for pigs that received PMR versus COM during the suckling period ($P < 0.05$), while intermediate values were observed for LMR and NO; relative empty stomach weight was not influenced by nursery treatment. Relative large intestine weight was not influenced by creep feed treatment, but was greater for pigs that received the LOW versus the HIGH nursery diet ($P < 0.05$). Relative liver weight was greater for pigs that received PMR versus those that received NO creep feed during the suckling period ($P < 0.05$), while intermediate values were observed for COM and LMR. Relative liver weight was greater for pigs that received the LOW versus HIGH nursery diet ($P < 0.001$).

3.4.4 Apparent Nutrient and Energy Digestibility After Weaning

In phases I and III, the AID of OM and CP and the ATTD of GE (phase III only) were not influenced by creep feed or nursery diet treatments (Table 3.8). In phase III, the ATTD of OM tended to be less for pigs that received the LOW versus HIGH nursery diet ($P = 0.076$) but was not influenced by creep feed treatment.

3.5 Discussion

The purpose of the current study was to determine the effects of creep feed composition and form on pig growth performance pre- and post-weaning and the utilization of low-complexity nursery diets. Creep feed form was the most important factor influencing DM intake and growth during the suckling period since LMR was consumed in greater quantities than PMR on a DM basis, despite having similar ingredient and nutrient compositions, which resulted in improved pre-weaning ADG and BW at weaning. Piglets fed LMR also had greater DM intake and BW at weaning than those fed a commercial (pelleted) creep feed. Moreover, BW at weaning was less for piglets that received PMR versus piglets that received LMR and COM and not different from piglets that received no creep feed. This was despite a lack of difference in ADFI and appearance of blue feces between PMR-fed piglets and COM-fed piglets, which could be due to poor pellet quality of the PMR (feed wastage) and binary classification (i.e. yes/no) versus quantification of blue dye appearance in feces, respectively. Finally, the greater BW at weaning for LMR-fed piglets did not translate into improved growth performance in the nursery period, regardless of nursery diet complexity. This is in contrast to the work of others that demonstrated improvements in nursery (e.g., Kim et al., 2001; Sulabo et al., 2010) and grower/finisher (Wolter et al., 2002) growth performance when pigs were offered milk replacer during the suckling period. In the current study, the familiarity with consuming feed (liquid or pellets) prior to weaning also did not reduce the latency to access nursery diet or apparent feed intake immediately after weaning. In addition, the use of creep feeds did not influence or minimize sow BW change, back fat and loin depth loss, or feed intake during the lactation period. Therefore, the provision of creep feed did not rescue sow BW loss during lactation or have extended benefits for the pigs in the post-weaning period.

It should be noted that the amount of LMR provided per piglet was limited by the reservoir capacity of the feeders, as well as labor requirements for mixing and delivering the milk replacer. By the end of the suckling period, the amount of LMR provided was capped at 3.0 L (as-fed; 0.75 kg of milk replacer powder) per litter per day. Furthermore, the ADG between days 18 and 21 of age was not different among creep feed treatment groups. If LMR had been provided *ad libitum*, it is possible that benefits for the sow during lactation and the piglets after weaning would have been apparent. Previous studies only noted a reduction in sow backfat loss when each piglet consumed an additional 10 g per day of the milk replacer powder than what was observed in the current study (Novotni-Dankó et al., 2015). Therefore, it is suggested that a more frequent feeding schedule or a liquid feeding system should be used to maximize litter LMR intake.

In the current study, pigs fed low complexity nursery diets that contained corn and soybean meal as the main protein sources had lower ADG, ADFI, and G:F in the early nursery period and were unable to exhibit compensatory growth to achieve BW equivalent to those of pigs fed high complexity nursery diets by the end of the nursery period. This is in contrast to the results of others (e.g., Huber et al., 2018; Lafleur Larivière et al., 2021), though in some cases, compensatory growth was not achieved until the end of the grower period (e.g., Skinner et al., 2014). It is noteworthy that the piglets used in the current study were exclusively from first parity sows and had lower BW at weaning (regardless of creep feed treatment) than the aforementioned studies that demonstrated compensatory growth for pigs fed low complexity nursery diets. Typically, offspring from first parity sows have poorer growth performance after weaning and until market weight, which is partly attributed to lighter birth weights and reduced immunoglobulin levels in colostrum and milk of first compared to multi-parity sows (Miller et al., 2012; Piñeiro et al., 2019). However, previous work demonstrated that gilt progeny provided with milk replacer in the suckling period did not achieve a greater BW than gilt progeny that were not provided milk replacer by 10 weeks of age (Miller et al., 2012). It is unknown whether the lower birth and weaning weights of first-parity offspring (versus offspring from multiparous sows) influence the ability to achieve compensatory growth after a post-weaning nutritional challenge.

Providing supplemental nutrients during the suckling period has been previously shown to benefit intestinal morphology in terms of greater villus height and reduced crypt depth for pigs four days after weaning (Zijlstra et al., 1993), which can improve nutrient absorption (Cera et al., 1988). Thus, it was hypothesized that creep feed or milk replacer intake during the suckling period would improve nutrient and energy digestibility in the nursery period. In the current study, there were no differences in AID or ATTD of OM, CP (AID only), or GE (ATTD only) related to creep feed treatment either at 28 (end of nursery phase I) or 59 (end of nursery phase III) days of age. In contrast, the AID of OM and CP at 28 days of age were 7 and 5 % less, respectively, for pigs that received the LOW versus HIGH nursery diet. Therefore, it appeared that the low complexity diet (i.e. with protein supplied mainly by corn and soybean meal) was less digestible by the small intestine early in the nursery period than the high complexity diet (i.e. with highly digestible animal protein sources). ATTD was not influenced by nursery diet treatment, despite pigs that received the LOW nursery diet having greater relative visceral organ weights by 59 days of age (versus HIGH). Indeed, previous work has shown that pigs develop larger visceral organs to utilize less digestible diets (Pluske et al., 2003). However, visceral organs also increase energy requirements for maintenance and reduce carcass value at slaughter, both of which negatively impact profitability (Nyachoti et al., 2000). Determining whether the greater visceral organ weights were maintained until market weight was beyond the scope of the study, but it is important that both creep and nursery feeding regimens promote adequate development of the GIT and nutrient utilization for protein deposition in the carcass, without disproportionally increasing visceral mass (Nyachoti et al., 2000).

In the current study, LMR was the most expensive creep feeding option in terms of cost per pig and cost per kilogram BW at weaning, which was largely driven by differences in apparent intake among the creep feed treatments. Moreover, these cost estimates do not account for the additional labor or specialized feeding systems required to provide liquid creep feeds. Despite heavier BW at weaning, providing LMR did not translate into more efficient use of the nursery diets and subsequent feed cost savings. Conversely, and regardless of creep feeding treatment, providing a low complexity nursery diet resulted in approximately $4.30 feed cost savings per pig and $0.10 per kilogram of (nursery) exit body weight. Therefore, the improvement in growth performance for pigs fed the high complexity nursery diet was not proportionally increased with greater feed cost.

3.6 Conclusion

In summary, providing LMR during the suckling period increased piglet BW at weaning, with no apparent benefit for the sow. Furthermore, supplying creep feed during the suckling period had limited impact on the utilization of nursery diets but pigs that received high complexity nursery diets had improved growth performance, particularly early after weaning. However, regardless of creep feeding regimen, low complexity diets are a means to reduce nursery feed cost. Therefore, based on the current study, nursery diets influence post-weaning growth performance to a greater extent than creep feeding regimen. Additional research is required to determine the effects of creep feeding strategy and nursery diet complexity for primiparous offspring on compensatory growth during the grower/finisher phase, in addition to days-to-market and carcass quality.

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Table 3.1: Calculated and analyzed nutrient contents (as-fed basis) of commercial creep feed (COM), liquid milk replacer (LMR), and pelleted milk replacer (PMR)

Item	COM[1]	LMR[2]	PMR[2]
Guaranteed analysis, as-fed			
NE, kcal/kg	2541	2903	2903
Crude protein, %	22	22	22
Calcium, %	0.9	0.9	0.9
Phosphorus, %	0.8	0.8	0.8
Total Lys, %	1.7	1.7	1.8
SID Lys, %[3]	1.5	1.6	1.6
Analyzed nutrient content, % (as-fed)			
Dry Matter	92.14	93.01	92.84
Crude protein	23.24	23.09	21.38
Calcium	0.78	0.86	0.81
Phosphorus	0.73	0.80	0.80
Potassium	1.17	1.49	1.42
Magnesium	0.12	0.08	0.08
Sodium	0.43	0.57	0.48

[1] Commerical creep feed from Floradale Feed Mill (Floradale, ON, Canada).

[2] Liquid milk replacer (powder) and pelleted milk replacer from Grober Nutrition (Cambridge, ON, Canada).

[3] Standardized ileal digestible.

Table 3.2. Ingredient composition and calculated and analyzed nutrient contents of nursery diets (as-fed basis)[1]

	HIGH			LOW		
Item	Phase I	Phase II	Phase III[2]	Phase I	Phase II	Phase III[2]
Ingredient, % (as-fed)						
Corn	16.8	37.78	49.59	46.42	49.37	47.2
Soybean meal, dehulled	13	16	22	24	34	37
Wheat	-	-	-	10	10	10
Barley	25	25	20	-	-	-
Fat, animal vegetable blend	2.5	2.5	2.5	2.5	2.5	2.5
Herring meal	5	3	-	5	-	-
Blood plasma[3]	4.5	2	-	-	-	-
Blood meal, spray dried	-	2	2	-	-	-
Oat groats	10	-	-	-	-	-
Whey	20	8	-	8	-	-
L-Lysine·HCl	0.35	0.33	0.38	0.47	0.35	0.1
DL-Methionine	0.10	0.16	0.14	0.06	0.11	-
L- Threonine	0.05	0.13	0.14	0.13	0.09	-
L- Tryptophan	-	0.03	0.05	0.02	-	-
Limestone	1	1.02	1.1	1	1.18	1.1
Salt	-	0.2	0.3	0.2	0.4	0.3
Monocalcium phosphate	0.8	1.25	1.2	1.3	1.4	1.2
Vitamin and mineral premix[4]	0.6	0.6	0.6	0.6	0.6	0.6
Titanium dioxide	0.3	-	-	0.3	-	-
Calculated nutrient composition, as-fed[5]						
NE, kcal/kg	2588	2530	2500	2557	2489	2475
Crude protein, %	21.5	20.4	19.2	21.3	21.9	22.7
Total Lys, %	1.58	1.45	1.34	1.55	1.44	1.34
SID Lys, %[6]	1.40	1.29	1.19	1.38	1.29	1.17
Calcium, %	0.89	0.85	0.81	0.91	0.87	0.85
Phosphorus, %	0.76	0.75	0.67	0.78	0.75	0.73
Analyzed nutrient composition, %[7]						
Crude protein	22.5	20.6	19.9	21.5	22.4	22.0
Calcium	0.80	0.86	0.75	0.76	0.78	0.78
Phosphorus	0.61	0.80	0.65	0.68	0.70	0.67

[1] Dietary treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[2] Phase III diets contained 0.2% titanium dioxide at the expense of corn during the final ten days.

[3] AP920; manufactured by APC Nutrition Inc. (Ames, IA).

[4] Provided, per kilogram of diet, 12,000 IU vitamin A as retinyl acetate, 1,299 IU vitamin D3 as cholecalciferol, 48 IU vitamin E as dl-α-tocopherol acetate, 3 mg vitamin K as menadione, 19 mg pantothenic acid, 6 mg riboflavin, 600 mg choline, 2.4 mg biotin, 18 mg Cu from CuSO4·5H2O, 120 mg Fe from FeSO4, 24 mg Mn from MnSO4, 126 mg Zn from ZnO, 0.36 mg Se from Na2SeO3, and 0.6 mg I from KI (DSM Nutritional Products Canada Inc., Ayr, ON, Canada).

[5] Calculated based on the NRC (2012) ingredient values. For Phase III -/+ titanium dioxide values are calculated on a weighted average basis using the number of days each diet was fed.

[6] Standardized ileal digestible.

[7] Phase III diet analyzed nutrients expressed as a weighted average between Phase III -/+ titanium dioxide fed for 7 and 10 days, respectively.

Table 3.3. Effect of creep feed form and composition on sow and piglet performance during lactation

	Treatment[1]					
	COM	LMR	PMR	NO	SEM[2]	*P*-value
No.[3]	14	14	14	14		
Sow performance						
Initial body weight, kg	183.8	192.5	196.3	188.8	7.1	0.623
Change in BW, kg[4]	-10.0	-8.8	-7.8	-9.9	3.2	0.443
Change in backfat, mm[5]	-2.2	-2.5	-2.7	-2.7	0.7	0.916
Change in loin depth, mm[5]	-1.2	-3.5	-4.2	-4.5	1.3	0.250
Average daily feed intake, kg[6]	6.9	6.4	6.5	6.9	0.5	0.909
Estrus interval, days	4.9	4.9	5.0	5.2	0.3	0.857
Piglet performance						
Litter size	11.9	11.7	11.6	11.9	0.2	0.708
Initial BW, kg[7]	2.45	2.34	2.35	2.36	0.08	0.268
Average daily gain, g	268^{a}	280^{a}	248^{b}	260^{ab}	10	<0.001
Average daily creep feed disappearance, g[8]	132^{b}	452^{a}	127^{b}	-	18	<0.001
Weaning BW, kg	6.02^{b}	6.33^{a}	5.66^{c}	5.92^{bc}	0.14	<0.001
Creep feed cost, $/pig	0.31^{b}	2.33^{a}	0.38^{b}	-	0.06	<0.001
Creep feed cost, $/kg BW at weaning	0.05^{b}	0.39^{a}	0.07^{b}	-	0.04	<0.001

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer (LMR), and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±0.3 days of age and until weaning (21.3±2.1 days of age).

[2] Maximum value for the standard error of the means.

[3] Number of sows or litters.

[4] Sow BW was measured within 24 hours of farrowing and at weaning (21 ±2.1days).

[5] Backfat and loin depth were measured on day 110 of gestation and at weaning.

[6] Sows were fed a commercial lactation diet. Feed intake was recorded between the start of the creep feed treatment until weaning.

[7] Initial piglet BW was recorded upon initiating creep feed treatments.

[8] Average daily creep feed disappearance per litter (DM-basis) for the creep feeding period.

[a, b] Within a row, means without a common superscript differ, $P < 0.05$.

Table 3.4. Effects creep feed form and composition on feed disappearance and the percentage of pigs consuming creep feed[1]

	Treatment[1]				
Item	COM	LMR	PMR	SEM[2]	*P*-value
No.[3]	14	14	14		
Average daily creep feed disappearance, g/pig[4]					
Day 5 to 9	4.3 ^{b}	18.8 ^{a}	4.3 ^{b}	0.8	<0.001
Day 10 to 13	8.4 ^{b}	28.2 ^{a}	6.7 ^{b}	1.6	<0.001
Day 14 to 15	11.7 ^{b}	36.1 ^{a}	9.2 ^{c}	2.3	<0.001
Day 16 to 17	12.4 ^{b}	48.8 ^{a}	14.8 ^{b}	2.9	<0.001
Day 18 to 21	16.4 ^{b}	55.7 ^{a}	16.6 ^{b}	1.7	<0.001
Blue-positive feces, %[5]					
Day 9	10.9 ^{b}	63.8 ^{a}	16.1 ^{b}	3.8	<0.001
Day 13	51.5 ^{c}	86.9 ^{a}	65.4 ^{b}	3.9	<0.001
Day 15	64.9 ^{b}	86.3 ^{a}	73.2 ^{b}	3.6	<0.001
Day 17	70.1 ^{b}	91.3 ^{a}	75.0 ^{b}	3.5	<0.001
Day 21	77.3 ^{b}	96.9 ^{a}	85.3 ^{b}	3.3	<0.001

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer (LMR), and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±0.3 days of age and until weaning (21.3±2.1 days of age).

[2] Maximum value for the standard error of the means.

[3] Number of litters evaluated.

[4] Average daily creep feed disappearance per pig (DM-basis).

[5] Brilliant blue dye was included with the creep diets (1%, as-fed) and presence in feces was determined via visual inspection. Percent of pigs with blue feces within a litter.

[a, b] Within a row, means without a common superscript differ, $P < 0.05$.

Table 3.5. The effect of creep feed form and composition on latency between weaning and first meal and nursery feed intake for the first two days post-weaning

	Creep Treatment[1]					Nursery Treatment[2]			*P*-value[3]	
	COM	LMR	PMR	NO	SEM[4]	HIGH	LOW	SEM[4]	CREEP	NURSERY
No.[5]	14	14	14	14		28	28			
Latency, hr[6]	13.1	8.4	8.5	7.8	2.1	10.6	8.3	1.6	0.097	0.117
Apparent feed intake, g/pig/day[7]	253	285	253	237	30	261	253	20	0.584	0.771

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer (LMR), and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±0.3 days of age and until weaning (21±2.1 days of age).

[2] Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[3] *P*-values for the main effects of creep (CREEP) and nursery dietary treatment (NURSERY).

[4] Maxiumum value for the standard error of the means.

[5] Number litters evaluated.

[6] Latency between weaning and eating was measured for each pig within each pen. A feeding bout was noted when the pig's head was in the feeder for three or more seconds or was observed chewing.

[7] Apparent feed intake for pigs during the first two days post-weaning.

Table 3.6. The effect of creep feed composition and form and nursery diet complexity on growth performance of pigs after weaning

	Creep treatment[1]					Nursery treatment[2]			*P*-value[3]	
	COM	LMR	PMR	NO	SEM[4]	HIGH	LOW	SEM[4]	CREEP	NURSERY
No.[5]	14	14	14	14		28	28			
Body weight, kg[6]										
Day 21	6.02^{b}	6.33^{a}	5.66^{c}	5.92^{bc}	0.14	6.02	6.00	0.08	<.0001	0.364
Day 28	6.96^{xy}	6.97^{x}	6.66^{y}	6.80^{xy}	0.20	7.07	6.63	0.19	0.064	<0.001
Day 42	11.79	11.94	11.33	11.30	0.48	12.45	10.74	0.40	0.067	<0.001
Day 59	23.16^{xy}	23.45^{x}	22.47^{xy}	22.25^{y}	0.62	24.50	21.24	0.55	0.065	<0.001
ADG, g/kg of BW										
Phase I	15.7	13.6	17.7	14.5	1.8	19.0	11.7	1.5	0.166	<0.001
Phase II	29.0^{xy}	29.9^{x}	29.9^{xy}	28.0^{y}	0.8	30.8	27.5	0.6	0.077	<0.001
Phase III	27.3	26.9	27.6	27.4	0.7	27.3	27.3	0.6	0.425	0.808
Overall[7]	26.2	26.3	27.2	26.1	0.4	27.7	25.1	0.4	0.154	<0.001
ADFI, g/kg of BW										
Phase I	24.2^{ab}	25.9^{a}	23.9^{ab}	22.2^{b}	3.1	25.8	22.7	3.1	0.044	<0.001
Phase II	35.1^{y}	36.3^{y}	38.4^{x}	38.2^{x}	1.6	38.0	36.1	1.4	0.036	0.044
Phase III	36.5^{x}	33.5^{y}	35.9^{xy}	36.2^{xy}	1.1	35.2	35.8	0.9	0.046	0.462
Overall	34.2	34.1	35.7	35.3	1.3	35.2	34.4	0.7	0.328	0.326
G:F										
Phase I	0.78	0.62	0.80	0.59	0.13	0.77	0.62	0.12	0.093	0.031
Phase II	0.88	0.85	0.80	0.79	0.05	0.86	0.80	0.04	0.071	0.070
Phase III	0.77	0.81	0.79	0.78	0.02	0.79	0.78	0.02	0.541	0.685
Overall	0.79	0.78	0.78	0.76	0.03	0.81	0.75	0.02	0.764	0.001
Feed cost, $/pig										
Nursery	14.42	14.09	14.19	14.31	0.57	16.42	12.08	0.55	0.757	<0.001
Creep + Nursery	14.75^{b}	16.42^{a}	14.58^{b}	14.34^{b}	0.59	17.17	12.87	0.56	<0.001	<0.001

Total feed cost, $/kg exit BW^8	0.65^b	0.71^a	0.65^b	0.65^b	0.02	0.71	0.62	0.02	0.001	<0.001

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer (LMR), and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±0.3 days of age and until weaning (21.3±2.1 days of age).

[2] Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[3] *P*-values for the main effects of creep (CREEP) and nursery dietary treatment (NURSERY).

[4] Maxiumum value for the standard error of the means.

[5] Number litters evaluated.

[6] BW was influenced by the interaction of creep and nursery feed treatments; see figure 1.

[7] Overall ADG was influenced by the interaction of creep and nursery feed treatments; see figure 2.

[8] Sum of creep and nursery diets consumed per pig divided by nursery exit BW.

[a, b] Within a row, means without a common superscript differ, $P < 0.05$.

[x, y] Within a row, means without a common superscript tend to differ, $0.05 \leq P \leq 0.10$.

Table 3.7. The effect of creep feed composition and form and nursery diet complexity on relative organ weights of pigs after weaning

	Creep treatment[1]					Nursery treatment[2]			*P*-value[3]	
	COM	LMR	PMR	NO	SEM[4]	HIGH	LOW	SEM[3]	CREEP	NURSERY
No. [5]	14	14	14	14		28	28			
Live body weight, kg										
Day 21	6.32	6.29	6.00	6.58	0.49	-	-	-	0.711	-
Day 28†	7.17	7.17	6.77	6.88	0.23	7.36	6.64	0.16	0.495	0.002
Day 59†	23.64^{y}	23.80^{y}	21.50^{x}	22.76^{xy}	0.72	24.80	21.06	0.54	0.049	<0.001
Full gut, g/kg of BW										
Day 21	68.8	77.0	75.4	76.2	8.6	-	-	-	0.631	-
Day 28	157.5	272.4	154.9	204.8	48.4	209.2	185.6	34.6	0.292	0.629
Day 59	164.7^{ab}	158.2^{b}	176.5^{a}	160.7^{b}	5.9	159.5	170.7	2.9	0.014	0.008
Stomach, g/kg of BW										
Day 21	5.4	5.4	5.5	5.2	0.5	-	-	-	0.608	-
Day 28	7.1	6.9	7.4	7.3	0.2	7.2	7.2	0.2	0.326	0.919
Day 59	7.2^{b}	7.3^{ab}	7.8^{a}	7.7^{ab}	0.2	7.5	7.4	0.2	0.017	0.605
Small intestine, g/kg of BW										
Day 21	35.0	38.1	37.0	35.7	2.7	-	-	-	0.534	-
Day 28	46.7	48.5	47.1	46.8	0.4	47.8	46.8	1.4	0.751	0.448
Day 59	53.1^{xy}	51.2^{y}	55.4^{x}	52.4^{xy}	1.2	49.5	56.5	0.8	0.071	<0.001
Large intestine, g/kg of BW										
Day 21	9.1	10.0	9.9	9.7	1.1	-	-	-	0.562	-
Day 28	18.1	17.0	17.1	16.7	0.4	16.9	17.5	0.7	0.596	0.401
Day 59	21.6	21.0	21.8	21.1	0.8	20.7	22.1	0.4	0.680	0.010
Liver, g/kg of BW										
Day 21	24.2	24.7	24.6	23.4	1.4	-	-	-	0.443	-

Day 28	24.8	26.5	24.5	24.4	0.7	24.8	25.3	0.5	0.089	0.499
Day 59	30.2^{xy}	30.1^{xy}	31.7^{x}	29.3^{y}	0.9	28.6	32.1	0.5	0.067	<0.001

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer (LMR), and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±0.3 days of age and until weaning (21.3±2.1 days of age).

[2] Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[3] *P*-values for the main effects of creep (CREEP) and nursery dietary treatment (NURSERY).

[4] Maxiumum value for the standard error of the means.

[5] Number litters evaluated.

[a, b] Within a row, means without a common superscript differ, $P < 0.05$.

[x, y] Within a row, means without a common superscript differ, $0.05 \leq P \leq 0.10$.

† Significant interaction between the main effects of CREEP and NURSERY, $P < 0.05$.

Table 3.8. The effect of creep feed composition and form and nursery diet complexity on apparent ileal digestibility (AID) and apparent total tract digestibility (ATTD) of nutrients and energy after weaning

	Creep[1]					Nursery[2]			*P*-value[3]	
	COM	LMR	PMR	NO	SEM[4]	HIGH	LOW	SEM[4]	CREEP	NURSERY
No.[5]	14	14	14	14		28	28			
AID, %										
Phase I										
Organic matter	66.4	63.6	68.3	67.8	3.8	68.8	64.2	2.5	0.574	0.076
Crude protein	53.9	53.2	58.1	54.1	5.5	56.1	53.6	4.8	0.745	0.463
Phase III										
Organic matter	64.2	63.0	61.7	61.7	2.3	63.2	62.1	2.0	0.792	0.583
Crude protein	74.8	72.1	72.6	72.0	1.8	74.5	71.6	1.3	0.583	0.108
ATTD										
Phase III										
Organic matter, %	77.5	81.5	81.3	80.0	1.5	79.8	80.3	1.4	0.199	0.699
GE, %	78.4	82.3	80.8	82.5	1.7	80.6	81.5	1.3	0.180	0.536
DE, kcal/kg	3548	3723	3653	3729	73	3627	3700	52	0.199	0.255

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer (LMR), and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±0.3 days of age and until weaning (21.3±2.1 days of age).

[2] Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[3] *P*-values for the main effects of creep (CREEP) and nursery dietary treatment (NURSERY).

[4] Maxiumum value for the standard error of the means.

[5] Number litters evaluated.

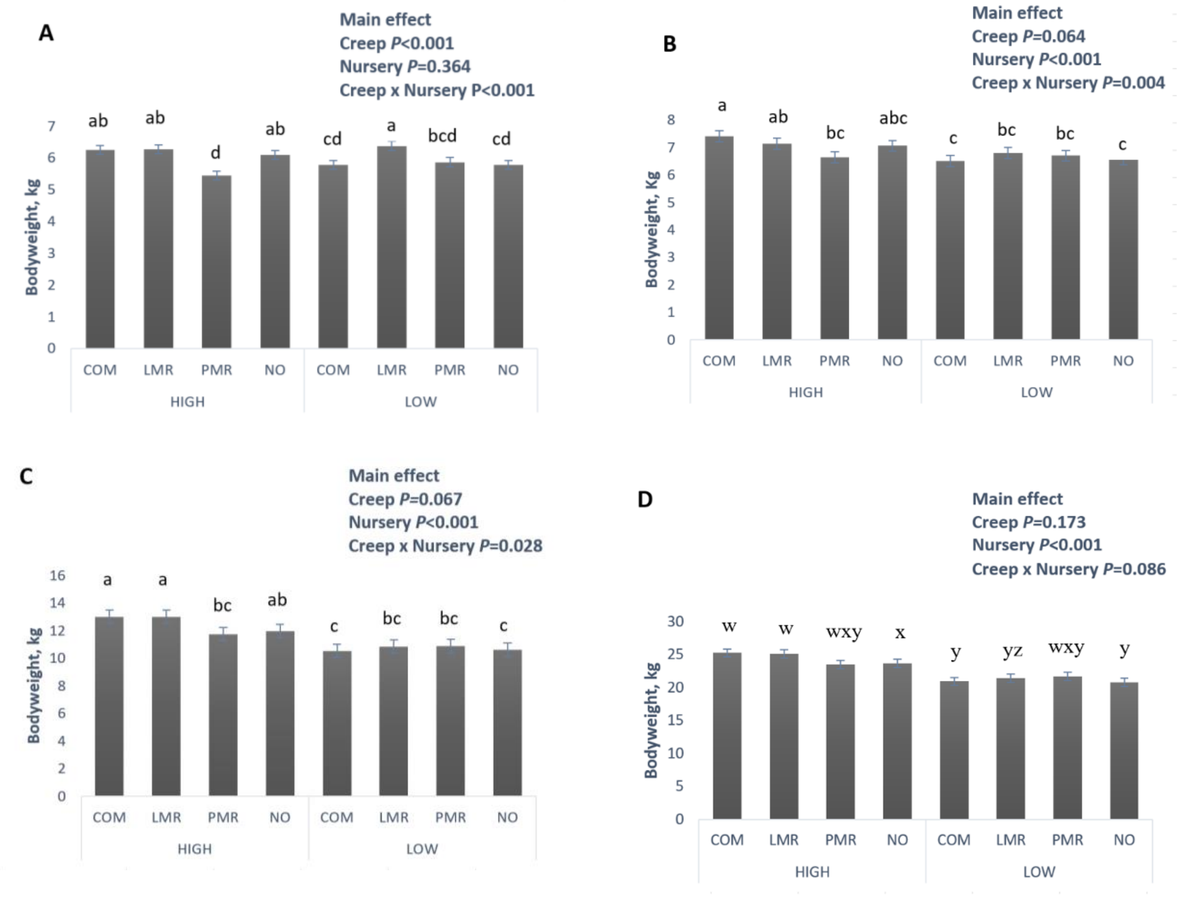

Figure 3.1. Interaction between creep and nursery feed treatments on bodyweight at (A) weaning (21 days of age), (B) 28 days of age, (C) 42 days of age and (D) 59 days of age. Creep feed treatments: commercial creep feed (COM), liquid milk replacer (LMR), pelleted milk replacer (PMR), or no creep feed offered (NO). Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources.

Values are Lsmeans ±SEM, n=7. Lsmeans without a common letter differ, $P < 0.05$

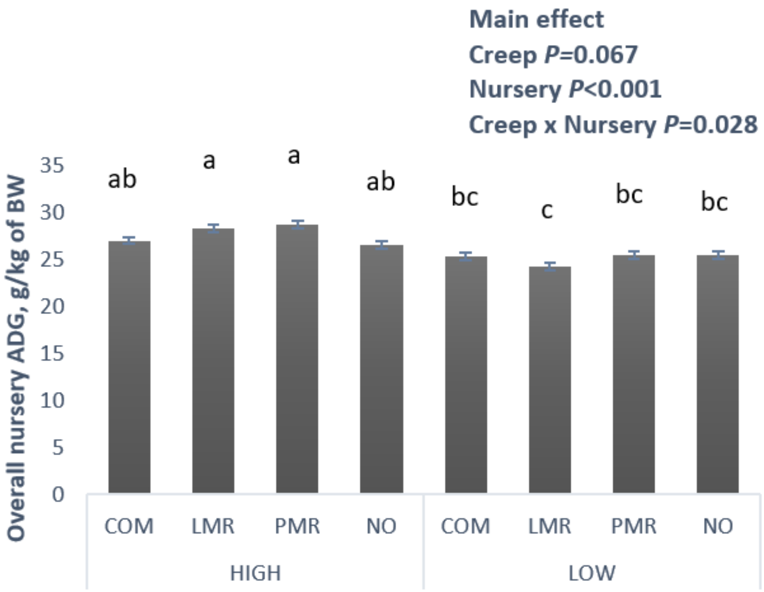

Figure 3.2. Interaction between creep and nursery feed treatments on ADG, g/kg of live BW throughout the nursery period. Creep feed treatments: commercial creep feed (COM), liquid milk replacer (LMR), pelleted milk replacer (PMR), or no creep feed offered (NO). Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources.

Values are presented as Lsmeans ±SEM, n=7. Lsmeans without a common letter differ, $P < 0.05$

CHAPTER 4: THE EFFECT OF CREEP FEED COMPOSITION AND FORM AND NURSERY DIET COMPLEXITY ON INTESTINAL MORPHOLOGY, JEJUNAL MUCOSA SPECIFIC ENZYME ACTIVITIES, AND IMMUNE RESPONSE OF PIGS

4.1 ABSTRACT

Fifty-six litters from first-parity sows standardized to 12 piglets were used to determine the effects of creep feed composition and form and the provision of low- or high-complexity nursery diets on intestinal morphology, specific enzyme activity and immune response post-weaning. At five days of age, litters (initial BW 2.31±0.61 kg) were assigned to one of four creep feeding regimens (n=14): [1] commercial creep feed (**COM**), [2] liquid milk replacer (**LMR**), [3] pelleted milk replacer (**PMR**), or [4] no creep feed (**NO**). At weaning (21 days of age), six pigs per litter that consumed creep feed were provided a **HIGH**- (contained highly digestible animal proteins) or **LOW**- (contained corn and soybean meal as main protein sources) complexity nursery diet (n=7) in a three-phase feeding program over 39 days. On 21 and 28 days of age, two pigs per pen (1 castrated male and 1 female) were slaughtered and jejunal mucosal scrapings were collected to determine specific mucosal enzyme activities. On day 28, jejunal and ileal samples were collected for histomorphological measurements. On days 29 and 43, two pigs per pen were vaccinated with ovalbumin (**OVA**) and 14 days post-injection, blood samples were collected to assess plasma concentration of OVA-specific immunoglobulin G (**IgG**). On day 57, these same two pigs underwent a dermal hypersensitivity response (**DHR**) test with skinfold thickness measured at 0, 6, 24, and 48 hr post-intradermal OVA injection. On day 28, pigs fed PMR had greater mucosal maltase specific activity compared to pigs that received NO and COM (456 vs. 576 and 463±34 U/mg of protein, respectively; $P < 0.05$ and $P = 0.071$) and pigs fed COM had lower mucosal specific lactase activity and jejunal absorptive capacity than pigs that received NO creep feed (88 vs. 50±11 U/mg of protein; $P = 0.05$; 8.74 vs. 7.45 ±0.48 μm^2; $P < 0.05$). Pigs given the LOW nursery diet had lower villus height (**VH**; 379 vs. 426±18 μm; $P < 0.05$), VH:crypt depth ratio (1.53 vs. 1.78±0.14; $P < 0.05$), and absorptive capacities in the jejunum (7.67 vs. 8.47±0.39 μm^2; $P < 0.05$) and ileum (7.54 vs. 8.24±0.25 μm^2; $P < 0.05$) than pigs fed HIGH nursery diet. On day 43, pigs fed COM tended to have lower plasma OVA-specific IgG concentration than pigs fed NO (0.18 vs. 0.38 ± 0.05 corrected optical density; $P = 0.07$) but there were no differences among treatment groups on day 57. Pigs fed PMR tended to have a smaller DHR than COM 6 hr after intradermal OVA injection ($P = 0.053$). Creep feeding did not alleviate post-weaning villus atrophy (absorptive capacity and specific enzyme activity) and had minimal effects on immune response at the end of the nursery period. Additionally, pigs provided a low-complexity diet had greater villus atrophy therefore reducing absorptive capacity than those provided a high-complexity nursery diet.

4.2 INTRODUCTION

During the suckling phase, milk production by the sow limits piglet growth, which is especially evident for first-parity sows and sows with large litters (Strathe et al., 2017). Additionally, after weaning, piglets experience a growth lag due to stressors including the abrupt change in diet composition and form (milk vs. cereal grains; liquid vs. pelleted) and the accompanying reduction in feed intake (Pluske et al., 2007; Sulabo et al., 2010b; Muns and Magowan, 2018). Conversely, piglets that consume creep feed or additional supplemental milk replacer during the suckling phase typically have a shorter fasting interval and increased feed intake after weaning, with resulting improvements in growth compared to those not provided creep feed (Bruininx et al., 2002; Miller et al., 2012). In many cases, the benefits of providing creep feed or additional milk replacer last for seven days after weaning, but in some cases, pigs that received supplemental feeds had greater ADG and ADFI for the entire nursery period versus pigs that did not receive supplemental feeds (Bruininx et al., 2002; Collins et al., 2013; Muns and Magowan, 2018). A shorter fasting interval and greater feed intake immediately after weaning can mitigate fasting-induced villus atrophy, crypt hyperplasia, and reduced absorptive capacity in the small intestine (Pluske et al. 1995). Moreover, despite some piglets sampling the sows' diet during the suckling period (Wattanakul et al., 2005), most piglets that are not offered creep feed have no exposure to plant-based ingredients and the accompanying adaptations to the digestive tract necessary to effectively process plant-derived components (Koo et al., 2017).

After weaning, the profile of digestive enzymes adjusts to accommodate changes in diet composition. For example, lactase is reduced and various pancreatic (e.g., trypsin, chymotrypsin, and amylase) and brush border (e.g., maltase and sucrase) specific enzyme activities increase (Jensen et al., 1997; Wattanakul et al., 2007; Tsukahara et al., 2013). Moreover, following weaning, an initial decline in digestive enzyme activity is observed, apart from lactase, which steadily declines post-weaning (Hampson and Kidder, 1986; Levesque et al., 2012). The adaptation of both pancreatic and brush border enzyme activities occurs during the first two weeks after weaning, after which the activities surpass pre-weaning levels (Hampson and Kidder, 1986; Jensen et al., 1997). This lag in digestive enzyme activities adaption is related to the post-weaning growth lag, with the greatest reduction in growth coinciding with the lowest enzyme activities (Hampson and Kidder, 1986).

Creep feed is typically provided to piglets to increase energy and nutrient intakes during the suckling period (Bruininx et al., 2002) and to stimulate the production of carbohydrate-degrading enzymes to assist the gastrointestinal tract in adapting to cereal (de Passillé et al., 1989). Nursery diets are typically formulated to be highly digestible by the immature gastrointestinal tract of the piglet by using animal-derived ingredients (e.g., lactose, whey, fishmeal etc.; Ma et al., 2019). Alternatively, soybean meal is a less expensive protein source, but is less digestible (Cervantes-Pahm and Stein, 2010) and contains antigenic compounds that can lead to intestinal inflammation (Koo et al., 2017; Ma et al., 2019; Koo et al., 2020). Previous work has shown that pigs can be fed corn- and soybean meal-based (low-complexity) diets immediately after weaning and, following an initial reduction in ADG, are able to achieve BW comparable to pigs fed highly digestible nursery diets (i.e. high-complexity; Skinner et al., 2014; Huber et al., 2018). It is not known whether providing creep feed during the suckling period will accelerate piglet gut maturation to minimize weaning- and soybean meal-induced damage to the gastrointestinal tract. Therefore, the objective of this study was to determine the effect of creep feed composition and form and nursery diet complexity on intestinal morphology, jejunal mucosal specific enzyme activities, and immune response of pigs after weaning.

4.3 MATERIALS AND METHODS

4.3.1 Animals, dietary treatments, and feeding

The experimental protocol was approved by the University of Guelph Animal Care Committee and followed Canadian Council on Animal Care guidelines (CCAC, 2009; AUP #4044). The study was conducted at the University of Guelph Arkell Swine Research Station (Guelph, ON, Canada).

Six hundred seventy-two piglets (Landrace × Yorkshire × Duroc) born to 56 first-parity sows over seven breeding batches (blocks) were used for the study. Litters were standardized to 12 piglets within 48 hours of parturition. At weaning, six piglets per litter (3 castrated males and 3 females) of median BW were selected to continue the study and were weaned into a nursery pen (one pen per litter); animal management and housing are described in Chapter 3.

Starting at 5 ± 0.3 days of age (initial BW 2.38 ± 0.02 kg) and continuing until weaning at 21 ± 2.1 days of age, litters were provided with one of four creep feed treatments according to a randomized block design: [1] commercial creep feed (**COM**), [2] liquid milk replacer (**LMR**), [3] pelleted milk replacer (**PMR**), or [4] no creep feed (**NO**; n=14). The COM contained corn and fishmeal with no milk products (Floradale Feedmill Ltd., Floradale, Ontario, Canada), while the LMR (powder) and PMR were formulated with matched levels of net energy, crude protein, crude fat, and standardized ileal digestible Lys supplied by milk products (Grober Nutrition, Cambridge, ON, Canada). All creep feeds and milk replacer powder contained 1% (wt/wt) brilliant blue dye for visual identification of individual piglets that consumed creep feed via appearance in the feces (Chapter 3).

At weaning, six pigs from each litter that were classified as ‘eaters’ (i.e. produced fecal swabs positive for blue dye for at least 2 days prior to weaning) were selected and placed in a nursery pen (1 pen per litter; pen was the experimental unit). Nursery pens were randomly assigned either a high- (**HIGH**; contained highly digestible animal protein sources) or low-complexity (**LOW**; contained corn and soybean meal as the main protein sources) nursery diet according to a 4×2 factorial arrangement (i.e. with creep feed treatment and nursery diet as the two factors; n=7).

Nursery diets were fed in a three-phase feeding program with phases I, II, and III fed for 7, 14, and 17 days, respectively.

4.3.2 Experimental Procedures

At weaning (21 days of age) and one-week post-weaning (28 days of age), two pigs per litter were randomly selected (1 castrated male and 1 female; 14 pigs per treatment) and were euthanized with an intra-cardiac injection of 3 mL of Euthasol (Virbac, TX). Immediately thereafter, the entire gastrointestinal tract was excised, and intestinal samples were collected. Mucosal scrapings from the center of the jejunum (20 cm) were collected by using a glass slide to separate mucosa from connective tissue. Samples were then flash frozen in liquid nitrogen and stored at -80°C until further analysis. An additional five-centimeter segment of the jejunum (approximately 1.5 m distal to the ligament of Trietz) and ileum (approximately 0.5 m proximal to the ileo-cecal junction) were collected, rinsed with physiological saline (0.9% NaCl), and stored in 10% formalin until further analysis. Jejunal and ileal tissue segments were prepared for histology analysis according to the procedures of Carleton et al. (1980). Measurements of villus height (**VH**), villus width (**VW**), crypt depth (**CD**), and crypt width (**CW**) were collected from 10 villi per pig in each intestinal section as shown in Figure 4.1 (Leica microsystems Inc., Wetzlar, Germany and Openlab Computer Imaging System; Perkin Elmer, Waltham, MA). The VH:CD and absorptive capacity (**M**) were calculated using the average values for each VH, VW, CW for each segment. Absorptive capacity (Eq. 1) was calculated according to Kisielinski et al. (2002):

[Eq. 1]

$$M = \frac{(VW \cdot VH) + (\frac{VW}{2} + \frac{CW}{2})^2 - (\frac{VW}{2})^2}{(\frac{VW}{2} + \frac{CW}{2})^2}$$

On days 29 and 43 of age, 2 pigs per pen (1 castrated male and 1 female; for blocks 1-3 and 6-7 only, due to COVID-19 restrictions) were randomly selected and immune-sensitized with 0.5 mg of ovalbumin (**OVA**) using 0.5 mg of Quil A as the adjuvant in 1 mL of saline (Sigma-Aldrich Co., St. Louis, MO) via intramuscular injection. Blood samples were collected via orbital-sinus puncture using plasma vacutainer tubes containing an anticoagulant (EDTA; BD Vacutainer®, BD, Franklin Lakes, NJ, USA) prior to each injection to measure the primary and secondary response via plasma OVA-specific immunoglobulin G (**IgG**). Blood samples were kept on ice and then were centrifuged for 15 min at 3,000 × g at 4°C. The resulting plasma was aliquoted into microcentrifuge tubes and stored at -20°C until analysis.

On day 57, all OVA-sensitized pigs were intradermally injected (0 hr) with 0.1 mL of (0.1mg/mL) OVA on the inner thigh of one hind leg. A control injection of 0.1 mL physiological saline was also injected at least 5 cm away from the OVA test site. Skin fold thickness was determined prior to initial injection in addition to 6-, 24- and 48 hr post-injection using calipers (Model RH15 9LB, Creative Health Products Inc., Ann Arbor, MI). The change in skin fold thickness (mm) over time relative to the saline injection measurement was used to determine the dermal hypersensitivity response (**DHR**):

[Eq. 2] DHR (mm) = $(\mathrm{SFT}_{\mathrm{time\ x}} - \mathrm{SFT}_{\mathrm{time\ 0}}) - (\mathrm{Saline}_{\mathrm{time\ x}} - \mathrm{Saline}_{\mathrm{time\ 0}})$, where $SFT_{time\ x}$ is the skin-fold thickness in mm at the OVA injection site at hr 0, 6, 24, or 48, $SFT_{time\ 0}$ is the skin-fold thickness in mm at the OVA injection site at hr 0, $Saline_{time\ x}$ is the skin-fold thickness in mm at the saline injection site at hr 0, 6, 24, or 48, and $Saline_{time\ 0}$ is the skin-fold thickness in mm at the saline injection site at hr 0.

The plasma OVA-specific IgG response was quantified using an indirect ELISA method as described by Begley et al. (2008), using a high-affinity binding 96-well microtiter plate (Corning, Acton, MA). The reference (plasma pooled from all pigs on day 43), quality control, and plasma samples were analyzed in triplicate, and optical density was measured at 405nm using the Epoch 2 microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT). The optical densities for individual plates were adjusted using a correction factor (**CF_{IgG}**; Eq. 3).

[Eq. 3]

$$\mathrm{CF_{IgG}} = \frac{\text{Overall mean of reference samples from all pigs}}{\text{Actual mean of individual plate reference sample}}$$

4.3.3 Enzyme Activity

Mucosal samples were collected with a glass slide scraping, and then flash frozen using liquid nitrogen then stored at -80°C. Mucosal samples were homogenized (1:20; w/v) using PowerGen 125 homogenizer (Fisher Scientific, Toronto, ON) in homogenization buffer (50 mM D-mannitol, 10mM Trizma·HCl and 10mM Hepes diluted in Milli-Q water and adjusted using 2.0 M NaOH to achieve pH of 7.4), and then aliquoted into microcentrifuge tubes and stored at -80°C until further analysis. Mucosal homogenates were analyzed for protein concentration in duplicate according to manufacturer instructions (Bio-rad, Hercules, CA); bovine serum albumin (Sigma

Chemical Company, St. Louise, MO) was used as the protein standard. Specific enzyme activities for sucrase (sucrose-isomaltase, E.C. 3.2.1.48), maltase (maltase-glucoamylase, E.C. 3.2.1.20), and lactase (lactase-phlorizin hydrolase, E.C. 3.2.1.108) were conducted according to Dahlqvist (1968). The reaction was stopped with BaOH and $ZnSO_4$ was used to precipitate the protein. Sucrase, maltase, and lactase specific activities were determined at 37°C for 10 min in a final volume of 200 μL using glucose oxidase as the reagent (Point Scientific Inc., Canton, MI) at substrate levels of 75 mM, 312.5 mM, and 312.5 mM for maltase, sucrase, and lactase, respectively. A lactase standard was included on each plate used to calculate a correction factor ($CF_{Lactase}$; Eq. 4) to adjust optical densities for individual plates. The optical density was measured at 500 nm with a reference wavelength of 650 nm. The amount of enzyme necessary to hydrolyze 1 nmol of the respective substrate hydrolyzed per minute at 37°C per mg of protein at a pH of 6.0 was defined as one unit.

[Eq. 4]

$$\mathrm{CF}_{Lactase} = \frac{\text{Overall mean of absorbance of lactase standard}}{\text{Absorbance of lactase standard on individual plate}}$$

4.3.4 Statistical Analysis

The statistical analyses for IgG, DHR, histomorphological measurements, and specific enzyme activities were conducted using the GLIMMIX procedure of SAS (University Edition; SAS Ins. Inc., Cary, NC) with creep feed treatment, nursery diet complexity, and the interaction between creep feed treatment and nursery diet complexity as the main effects and block as a random effect. For specific enzyme activities, IgG, and DHR, time was also included as a main effect and the interactions between time and other main effects were analyzed. The interaction between creep feed treatment and nursery diet was generally not significant unless stated otherwise, therefore only the main effects of creep feed treatments and nursery diet are presented. In all analyses, the degrees of freedom were calculated with Kenward-Roger's adjustment for repeated measures and outliers were detected using the univariate procedure. Model residuals were assessed using scatter and box plot of studentized residuals for homogeneity of variance, Q-Q plot and Shapiro-Wilk test for normal distribution. Mean comparisons were conducted using Tukey-Kramer post-hoc test to separate means. Probability (***P***)- values of less than 0.05 were considered significant, and $0.05 \leq P \leq 0.10$ were considered tendencies.

4.4 RESULTS

4.4.1 Specific Mucosal Enzyme Activities

Maltase, sucrase, and lactase activities in jejunal mucosa were not affected by creep feed treatment at weaning (21 days of age) nor by nursery diet at 28 days of age (Table 4.1). There was a tendency for an interaction between creep and nursery treatments for maltase activity at 28 days of age such that pigs that received PMR-LOW had greater specific maltase activity than pigs that received NO-LOW ($P = 0.092$; data not shown), with all other treatments being intermediate. At 28 days of age, jejunal mucosa maltase specific activity was greater for pigs fed PMR versus NO and tended to be greater for pigs fed PMR than for pigs fed COM ($P < 0.05$ and $P = 0.071$, respectively), while pigs fed LMR had intermediate maltase specific activity (Table 4.1). Sucrase specific activity was not affected by creep feed treatment at 28 days of age. Additionally, lactase specific activity tended to be greater for pigs that received NO creep feed treatment than for pigs fed COM ($P = 0.054$), while intermediate values were observed for LMR and PMR pigs (Table 4.1). Maltase specific activity tended to be influenced by the interaction of creep feed treatment and time such that specific activity tended to be greater at 21 days of age than at 28 days of age only for pigs that received COM (*P* = 0.085; Figure 4.2; Panel A). Additionally, the specific enzyme activity for sucrase and lactase were affected by time, such that the activity was reduced on day 28 compared to at weaning (*P* <0.001; Figure 4.2; Panel B and C).

4.4.2 Gastrointestinal Morphology

Creep feed treatment did not influence VH, VW, CD, CW, VH:CD in either the jejunum or ileum on day 28 of age (Table 4.2). Pigs that received the LOW nursery diet had smaller VH in both the jejunum and ileum versus pigs that received the HIGH nursery diet ($P < 0.05$ and $P = 0.090$, respectively). Although there were no differences in CD attributed to nursery treatment, the VH:CD ratio was less for pigs that received the LOW than for pigs fed HIGH nursery diets in both jejunum and ileum ($P < 0.05$). Pigs that received NO creep feed had greater absorptive capacity in the jejunum than pigs that received the COM diet ($P < 0.05$); pigs that received LMR and PMR had intermediate jejunal absorptive capacities. Pigs that received LOW nursery treatment had lower jejunal absorptive capacity than those that received the HIGH diet ($P < 0.05$). In the jejunum, pigs provided NO-HIGH tended to have greater absorptive capacity than LMR-LOW (*P* =0.075) and COM-HIGH (*P* =0.092; data not shown). Absorptive capacity in the ileum was also influenced by the interaction between creep and nursery treatments such that pigs that received NO-HIGH had greater absorptive capacity than pigs that received NO-LOW ($P = 0.013$) and tended to have greater absorptive capacity than pigs that received COM-LOW ($P = 0.062$; Figure 4.3) with all other treatments intermediate. Ileal absorptive capacity was not influenced by the main effect of creep treatment but tended to be less for pigs that received LOW versus HIGH nursery treatments ($P = 0.054$; Table 4.2).

4.4.3 Immune Response to OVA Vaccination

The plasma OVA-specific IgG concentration was greater on day 57 of age (secondary response) than on day 43 of age (primary response; *P* < 0.001; data not shown). On day 43, pigs that received the NO treatment tended to have greater plasma OVA-specific IgG concentration than pigs that received LMR and COM, regardless of nursery treatment (*P* = 0.020 and *P* = 0.028, respectively; Table 4.3), but no difference in secondary response due to creep feed or nursery treatments was observed.

The DHR to OVA challenge was influenced by the main effect of time after injection (*P* < 0.001) but not the main effects of creep feed treatment, nursery treatment, or the interactions between creep feed treatment or nursery treatment and time. Pigs that received the COM treatment in the suckling phase tended to have a greater change in skinfold thickness versus pigs that received PMR at 6 hr post-injection (*P* = 0.053), while intermediate values were observed for pigs that received LMR and NO creep feed treatments.

4.5 DISCUSSION

The objective of the current study was to determine the effect of creep feed composition and form and nursery diet complexity on intestinal morphology, specific mucosal enzyme activities, and immune response of pigs after weaning. Pigs provided a commercial creep feed experienced the greatest reduction in maltase specific enzyme activity and lower absorptive capacity in the jejunum (versus NO). Additionally, those provided a low-complexity diet had reduced absorptive capacity (versus HIGH), but nursery diet complexity did not affect specific enzyme activity or immune response. Therefore, creep feeding did not alleviate post-weaning gastrointestinal dysfunction or alter the immune response to vaccination. Nursery diet complexity was the main influencer of post-weaning gut morphology, as those provided a high-complexity nursery diet had greater absorptive capacity than those fed a low-complexity diet, regardless or creep feeding treatment.

Maintenance of gastrointestinal architecture after weaning, specifically VH, is crucial, since the activity of brush border enzymes (including lactase and sucrase) increase between the crypt to apical villi (Fan et al., 2001). Therefore, the greater the severity of villus atrophy, the greater the reduction in brush border enzyme activity (Tsukahara et al., 2013). This was also seen in the current study, with NO creep feed pigs having improved absorptive capacity as well as, tending to have greater lactase specific activity on day 28 than pigs that received commercial creep feed (COM) during the suckling period. Since lactase specific activity was still relatively greater one week after weaning for pigs that did not receive creep feed versus pigs that received commercial creep feed (COM), it appears that the adaption of digestive enzymes to plant-based diets was also delayed. However, the relatively lower lactase specific enzyme activity did not correspond to greater maltase or sucrase activities for pigs that received COM during the suckling period. Previous research has shown that maltase and sucrase specific activities are related to gastrointestinal maturity (i.e. longer villi; Tsukahara et al., 2013), and BW, as heavier pigs have increased activity of these enzymes (de Passillé et al., 1989). Other researchers have reported however, that feed composition affected specific enzyme activity, for example noting a reduction in α-amylase and maltase specific activities when feeding diets with reduced starch content (Trevisi et al., 2005; Bikker et al., 2006). The significant reduction in maltase activity in conjunction with a reduced absorptive capacity one-week after weaning, suggests that pigs fed the commercial creep feed appeared to have the greatest impairment after weaning in terms of intestinal morphology and specific enzyme activity.

The maintenance of gastrointestinal morphology after weaning is vital for pigs to maintain nutrient absorptive capacity and therefore, feed efficiency. The stress associated with weaning leads to long fasting intervals and low feed intake resulting in villus atrophy and crypt hyperplasia (Boudry et al., 2004). In the previous study described in Chapter 3, pigs that were provided the commercial creep feed (COM) consumed their first meal after weaning 5.3 hr later than those not provided with creep feed during the suckling period. However, it should be noted that total feed intake during the first 48 hr after weaning was not different among creep feed treatment groups and, during phase I, pigs that received LMR had greater relative feed intake than pigs that did not receive creep feed, regardless of nursery diet complexity (Chapter 3). In the current study, the pigs that did not receive creep feed (NO) during the suckling period tended to have greater jejunal absorptive capacity than pigs that received a commercial creep feed (COM), contrary to expectations. However, it is possible that the weaning- and fasting-induced reduction in absorptive surface area (Boudry et al., 2004; Heo et al., 2018) was alleviated for pigs that received no creep feed during the sucking period since they consumed feed sooner after weaning than those provided COM. The importance of early feed intake after weaning versus overall feed intake (within the first 24hr following initial feed intake post-weaning; Bruininx et al., 2002) is not known for gut physiology or morphology; therefore, additional research should be done to determine the correlation between post-weaning fasting intervals and maintenance of intestinal morphology.

Pigs fed a high-complexity nursery diet that included digestible animal-based protein sources had longer villi in the jejunum and ileum resulting in a greater VH:CD ratio and absorptive capacity in both segments versus those fed a low-complexity corn-and-soybean meal-based nursery diet. It is likely that the higher inclusion of soybean meal in the low-complexity diets (Chapter 3) was responsible for villus atrophy soon after weaning, as others observed a relationship between increasing soybean meal inclusion and shorter villi (Ma et al., 2019; Koo et al., 2020). Furthermore, pigs not provided creep feed were most affected by nursery diet complexity as NO-HIGH had greater absorptive capacity than NO-LOW. Moreover, when pigs were provided a commercial creep feed (COM), which was most similar to the nursery diet, they were better able to maintain absorptive capacity than those not provided creep feed when weaned onto a low-complexity nursery diet. This is further supported by pigs provided NO-HIGH having greater BW on days 43 and 59 of age than those provided NO-LOW, and by pigs provided HIGH nursery diets tending to have greater organic matter digestibility on day 28 than pig provided LOW diets (Chapter 3). Despite the VH being related to mucosal specific enzyme activities, as discussed above, no differences attributed to nursery diet complexity were observed for any of the enzymes examined in the current study. It can take up to 15 days for specific maltase activity to surpass pre-weaning activity and 7 days could be too short of an interval to see an effect of nursery diet complexity on enzyme specific activity after weaning (Hampson and Kidder, 1986; Boudry et al., 2004). The results for mucosal specific enzyme activities in the current study are more likely related to stress around weaning (acute phase), and not the recovery of pigs post-weaning (adaptive phase). Although the HIGH-complexity nursery diet had greater calculated absorptive capacity, no differences were observed in mucosal specific enzyme activities, it is possible this is the reason no differences in nutrient digestibility (Chapter 3) were observed. In Chapter 3, it was determined that pigs fed the high-complexity nursery diet had improved ADG, ADFI and G:F only during phase I and phase II of the nursery, and it is likely that by phase III, pigs were fully adapted to the nursery diets, in addition to the phase III diets being quite similar between the nursery diet treatments. Pigs provided a high-complexity nursery diet experienced less initial villus atrophy than those provided a low-complexity diet. Additionally, after showing initial differences in growth performance and feed efficiency, pigs provided the low-complexity diet adapted (e.g., heavier visceral organs top achieve similar nutrient digestibility) by the end of the nursery phase and achieved similar feed efficiency and nutrient digestibility as those given a high-complexity diet in phases I and II. It is likely that by the end of the nursery period gut morphology would also be restored for those provided the low-complexity diet.

The plasma OVA-specific IgG concentration was greater on day 57 than on day 43 of age (14 and 28 days after primary and booster immunizations against OVA), indicating the immunization protocol was successful, which was similar to what was observed by Crosbie et al. (2021). On day 43, pigs that received no creep feed during the suckling period had greater plasma OVA-specific IgG concentration versus those that received the commercial creep feed (COM) and LMR. However, there were no differences in plasma OVA-specific IgG concentrations on day 57 attributed to creep feeding regimen. Moreover, all pigs immunized against OVA had a positive DHR to the OVA antigen, with the greatest DHR observed 6 hr post-injection, which was expected for this antigen (Crosbie et al., 2021). However, there was no difference in DHR attributed to creep feed treatment. Finally, it was expected that differences between nursery treatments in OVA-specific IgG and DHR would be observed due to the antigenic compounds present in soybean meal (Koo et al., 2020). Other studies have observed no changes in DHR (Giesting et al. 1986; Huber et al., 2018), or lipopolysaccharide challenge (Dritz et al., 1996) attributed to soybean meal exposure. However, by phase III, the nursery diet ingredient composition was similar between the HIGH- and LOW- complexity diets. It is possible that differences in immune response would be observed if tested at an earlier age when pigs were being sensitized to soybean meal. In this current study, by the time the DHR test was being conducted, pigs consuming the low-complexity diet were likely no longer responding to the antigenic compounds in soybean meal.

4.6 CONCLUSION

Overall, pigs that were provided COM experienced the greatest reduction in jejunal maltase specific enzyme activities between weaning and one-week post-weaning and had lower absorptive capacity than those not provided creep feed by 28 days of age. Moreover, those not provided creep feed during the suckling period tended to have lower ileal absorptive capacity than those who were provided COM when given a low-complexity diet post-weaning. Although COM pigs had the greatest acute response to weaning, by the end of the nursery period they were able to mount an immune response like the other creep feed treatments, with similar AID and ATTD. Additionally, the pigs provided a high-complexity nursery diet had greater absorptive capacity showing that the low-complexity diet caused a greater degree of villus atrophy. However, no effects on specific enzyme activities or immune response were observed. Therefore, creep feeding did not mitigate the effects of weaning on gastrointestinal structure, or immune response, and when pigs were provided a high-complexity nursery diet they had improved gastrointestinal morphology compared to those provided a low-complexity nursery diet.

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Table 4.1. Effect of creep feed composition and form and nursery diet complexity on jejunal mucosa digestive enzyme specific activities for 21- (weaning) and 28-day old pigs.

	Creep Treatment[1]					Nursery Treatment[2]			P-Value[3]	
Item	COM	LMR	PMR	NO	SEM[4]	HIGH	LOW	SEM[4]	CREEP	NURSERY
No.[5]	14	14	14	14		28	28			
Maltase, U/mg of protein										
Day 21	660	517	522	545	78	-	-	-	0.347	-
Day 28	$463^{ab, y}$	559^{ab}	$576^{a, x}$	456^{b}	34	527	500	25	0.015	0.395
Sucrase, U/mg of protein										
Day 21	464	341	415	354	70	-	-	-	0.523	-
Day 28	118	201	196	165	32	169	171	22	0.211	0.964
Lactase, U/mg of protein										
Day 21	247	228	209	257	34	-	-	-	0.606	-
Day 28	50^{y}	59^{xy}	79^{xy}	88^{x}	12	73	66	9	0.043	0.551

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer, and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±2.1 days of age and until weaning (21.3±2.1 days of age).

[2] Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[3] *P*-values for the main effects of creep (CREEP) and nursery dietary treatments (NURSERY).

[4] Maximum value for the standard error of the means.

[5] Number of litters evaluated.

[a, b] Within a row, means without a common superscript differ, $P < 0.05$.

[x, y] Within a row, means without a common superscript tend to differ, $0.05 < P < 0.10$.

‡ Trend for the interaction between CREEP and NURSERY treatments, $0.05 < P < 0.1$.

Table 4.2. Effect of creep feed composition and form and nursery diet complexity on ileal and jejunal morphology one week after weaning for pigs weaned at 21 days of age

	Creep Treatment[1]					Nursery Treatment[2]			*P*-Value[3]	
	COM	LMR	PMR	NO	SEM[4]	HIGH	LOW	SEM[4]	CREEP	NURSERY
No.[5]	14	14	14	14		28	28			
Jejunum										
Villus height, µm †	375	384	424	427	24	426	379	18	0.131	0.015
Villus width, µm	105	105	110	108	5	105	109	4	0.686	0.318
Crypt depth, µm	272	245	250	272	19	254	266	15	0.397	0.398
Crypt width, µm	42	45	44	42	2	44	43	2	0.334	0.201
VH:CD	1.51	1.69	1.72	1.70	0.17	1.78	1.53	0.14	0.508	0.020
Absorptive capacity, µm^2 ‡	7.45^{y}	7.84^{xy}	8.25^{xy}	8.74^{x}	0.48	8.47	7.67	0.39	0.057	0.021
Ileum										
Villus height, µm	427	395	386	402	22	417	388	17	0.378	0.090
Villus width, µm	112	115	111	125	9	113	119	8	0.138	0.181
Crypt depth, µm	275	271	269	252	17	258	275	14	0.635	0.189
Crypt width, µm	48	45	47	45	2	46	46	2	0.327	0.666
VH:CD	1.61	1.51	1.56	1.73	0.10	1.73	1.48	0.08	0.324	0.004
Absorptive capacity, µm^2 †	8.26	7.88	7.66	7.75	0.37	8.24	7.54	0.25	0.659	0.054

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer, and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±2.1 days of age and until weaning (21.3±2.1 days of age).

[2] Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[3] *P*-values for the main effects of creep (CREEP) and nursery dietary treatments (NURSERY).

[4] Maximum value for the standard error of the means.

[5] Number of litters evaluated.

[a, b] Within a row, means without a common superscript differ, $P < 0.05$.

† Interaction between the main effects of CREEP and NURSERY, $P < 0.05$.

‡Trend in the interaction between the main effects of CREEP and NURSERY, $0.05 < P < 0.10$.

Table 4.3. Effect of creep feed composition and form and nursery diet complexity on plasma ovalbumin- (OVA) specific immunoglobulin G (IgG) response and the dermal hypersensitivity response (DHR) to OVA for pigs after weaning at 21 days of age

	Creep Treatment[1]					Nursery Treatment[2]			*P*-value[3]	
Item	COM	LMR	PMR	NO	SEM[4]	HIGH	LOW	SEM[4]	CREEP	NURSERY
No.[5]	9	12	12	9		21	21			
OVA-specific IgG										
Day 43	0.21[b]	0.21[b]	0.27[ab]	0.37[a]	0.06	0.28	0.25	0.05	0.015	0.386
Day 57	1.19	1.12	1.14	1.17	0.06	1.16	1.15	0.06	0.703	0.879
Changes in skinfold thickness, mm[6]										
Hour 0	0.08	-0.03	-0.05	-0.02	0.11	-0.00	-0.01	0.001	0.675	0.910
Hour 6	0.58	0.27	0.19	0.30	0.18	0.38	0.29	0.14	0.142	0.467
Hour 24	0.14	0.28	0.11	0.23	0.11	0.24	0.14	0.10	0.560	0.300
Hour 48	0.09	0.20	0.04	0.02	0.10	0.10	0.07	0.08	0.336	0.668

[1] Creep feed treatments: commercial creep feed from Floradale Feed Mill (Floradale, ON, Canada; micropellets; COM), liquid milk replacer, and pelleted milk replacer (micropellets; PMR) from Grober Nutrition (Cambridge, ON, Canada), or no creep feed offered (NO). Creep feed treatments were implemented at 5.4±2.1 days of age and until weaning (21.3±2.1 days of age).

[2] Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Diets were fed for 7, 14, and 17 days in phases I, II, and III, respectively.

[3] *P*-values for the main effects of creep (CREEP) and nursery dietary treatment (NURSERY).

[4] Maximum value for the standard error of the means.

[5] Number of litters evaluated.

[6] Number of hours after initial injection with 0.1mL of 0.1mg/mL of OVA antigen.

[a, b] Within a row, means without a common superscript differ, $P < 0.05$.

[x, y] Means without a common superscript tend to differ, $0.05 < P < 0.10$.

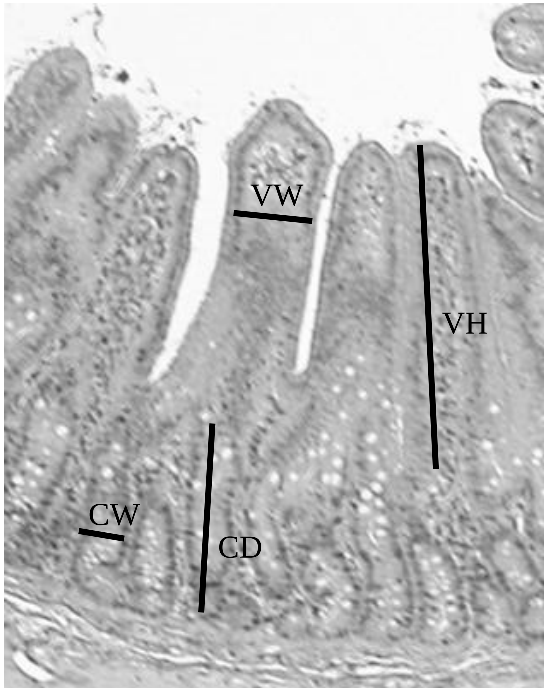

Figure 4.1. Example measurement of the four jejunal and ileal morphological parameters: villus height (VH), villus width (VW), crypt depth (CD) and crypt width (CW).

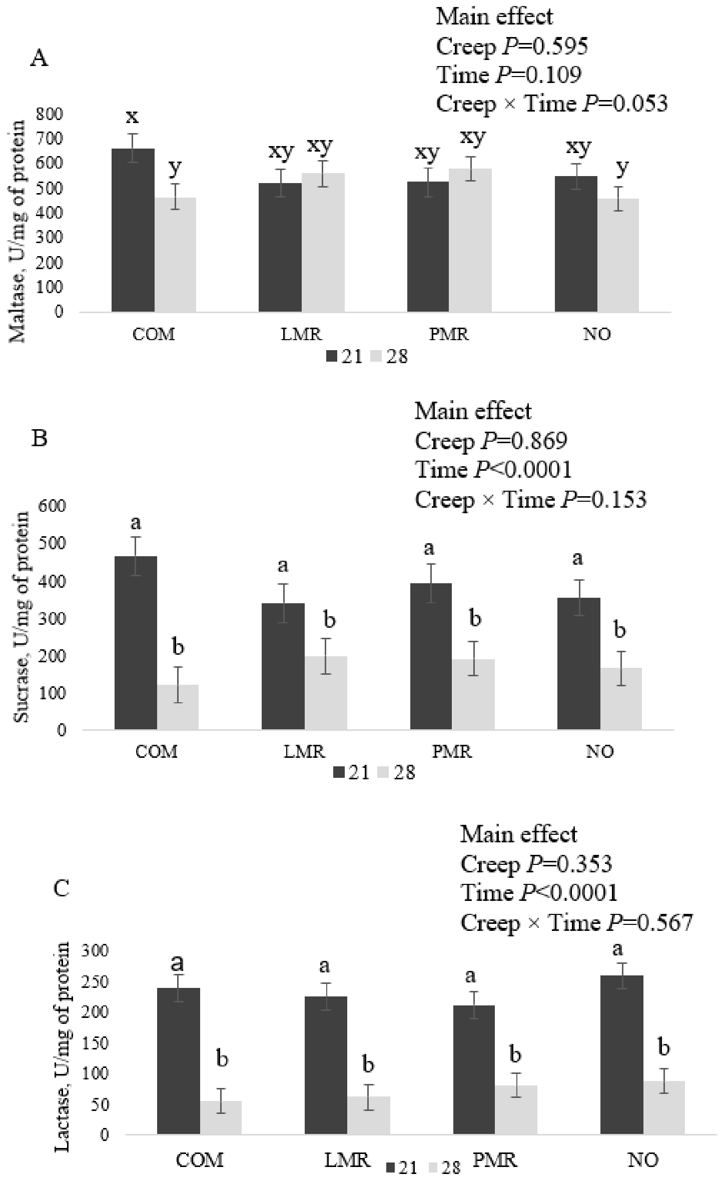

Figure 4.2. Specific activities of jejunal mucosa (A) maltase, (B) sucrase, and (C) lactase at weaning (day 21) and one-week post-weaning (day 28) for pigs fed either a commercial creep feed (COM), liquid milk replacer (LMR), pelleted milk replacer (PMR), or no creep feed (NO) during the suckling period. Values are Lsmeans ± SEM, n=14.

$^{a, b, c, d}$ Means without a common superscript differ, $P < 0.05$.

$^{x, y}$ Means without a common superscript tend to differ, $0.05 < P < 0.10$.

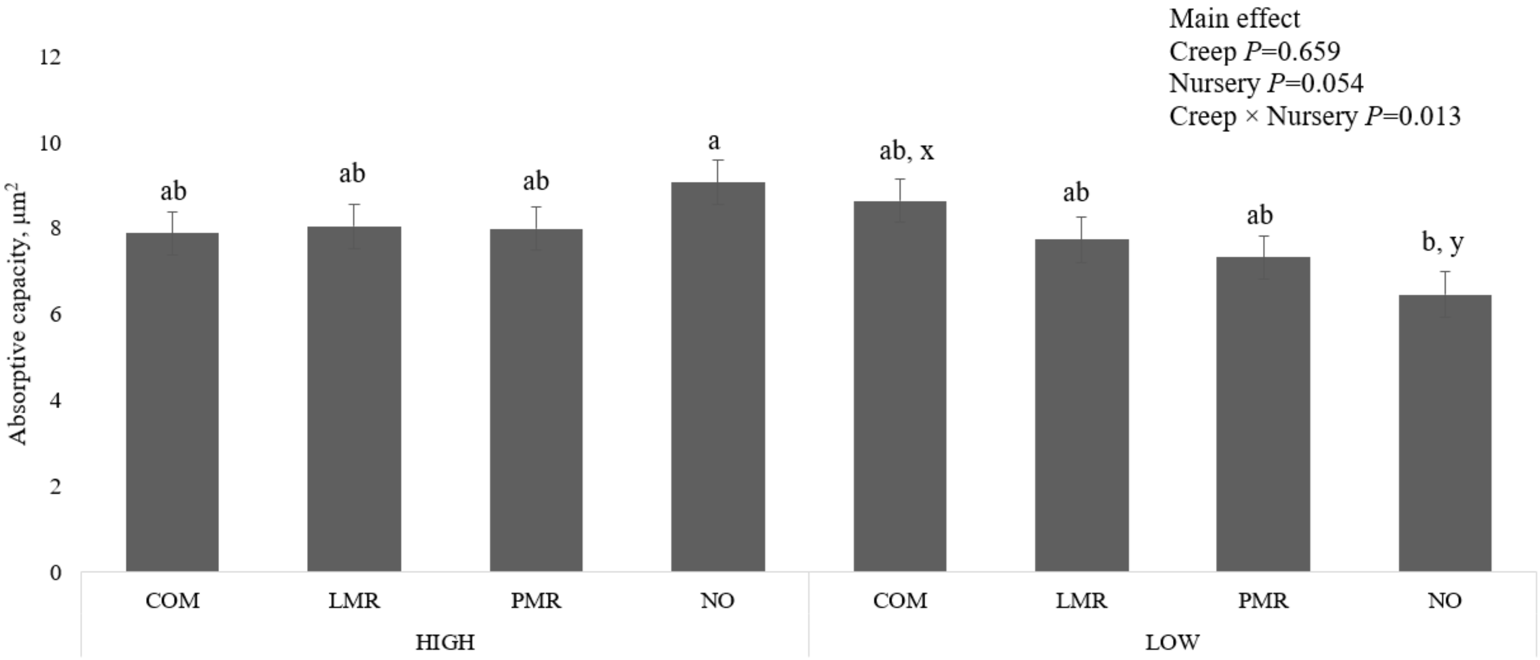

Figure 4.3. Effect of creep feed composition and form and nursery diet complexity on absorptive capacity (M) in the ileum for pigs at 28 days of age. Creep feed treatments: commercial creep feed (COM), liquid milk replacer (LMR), pelleted milk replacer (PMR), or no creep feed offered (NO). Nursery feed treatments: HIGH = nursery diets that contained protein from plant and animal sources; LOW = nursery diets with corn and soybean meal as the main protein sources. Values are Lsmeans±SEM, n=7.

$^{a, b}$ Means without a common superscript differ, $P < 0.05$.

$^{x, y}$ Means without a common superscript tend to differ, $0.05 < P < 0.10$.

CHAPTER 5: SUMMARY, GENERAL DISCUSSION, AND IMPLICATIONS

Research has shown that sows, particularly first-parity sows, cannot produce enough milk to support maximal piglet growth after 13 days of lactation (Strathe et al., 2017). Creep feed can be provided to piglets during the suckling phase to compensate for limiting milk intake. However, voluntary intake of creep feed remains relatively low throughout lactation and is highly variable between and within litters. Alternatively, milk replacer can be provided during this period, and is consumed more readily and consistently by piglets. Due to increased intake, milk replacer-fed piglets tend to be heavier at weaning than those fed pelleted creep feed, with piglets not provided any supplemental feed being weaned at the lowest body weight (Park et al., 2014; Agyekum et al., 2018; Heo et al., 2018).

After weaning, piglets experience a lag in growth due to change in environment, pen mates and feed composition and form (Colson et al., 2012). This lag in growth can last up to 2 weeks and has lasting effects on growth performance (i.e. ADG. ADFI, days to market) and gastrointestinal health (i.e. villus height, crypt depth, gut permeability; Lallès et al., 2007). Creep feed is one strategy that is used to reduce nutritional stress and promote feed intake after weaning (Park et al., 2014; Agyekum et al., 2018; Heo et al., 2018). Additionally, the ingredient composition of nursery diets has a major impact on subsequent growth performance (Skinner et al., 2014; Koo et al., 2020). Typically, piglets are provided a high-complexity diet (formulated with plant and animal protein sources), which has shown to improve growth performance immediately after weaning and throughout the nursery period (Skinner et al., 2014; Koo et al., 2020). However, these types of diet are expensive and rely on the availability of high-quality animal by-products (Skinner et al., 2014; Koo et al., 2020). Studies have shown that pigs provided a low-complexity diet after weaning (mainly corn and soybean meal) experience an initial growth lag but can catch-up in terms of body weight to pigs provided a high-complexity diet in a process known as compensatory growth (Skinner et al., 2014). Knowledge is limited regarding the interaction between pre- and post-weaning feed composition and form on growth performance and gut morphology and enzyme activity and immune response. The specific objectives of this thesis were: [1] determine which creep feed form (liquid vs. pelleted) and composition (corn and fish meal vs. whey and skim milk) on sow and piglet growth performance during the suckling and nursery phase and determine feed costs of creep and nursery treatments, [2] determine effects of creep feeding and nursery diet complexity on gut structure and function and immune system activation after weaning.

Providing creep feed did not affect any parameters measured regarding sow performance. However, as in previous studies, it seems that creep feed intake was too low in this study to observe any benefits to the sow (Novotni-Dankó et al., 2015). Piglet performance prior to weaning was greatly impacted by creep feed treatment. The LMR was consumed in the greatest quantity by the greatest proportion of piglets throughout the entire creep feeding period. Conversely, offering COM and PMR to piglets resulted in comparable but lower feed intakes and proportion of piglets consuming the feed throughout the entire creep feeding period, compared to offering piglets LMR. This shows that creep feed form and not composition affected feed intake. Consequently, LMR-fed piglets were weaned with the greatest BW, followed by COM and PMR, with NO being intermediate between PMR and COM. Although COM and PMR had similar feed intakes during the creep feeding period, PMR had lower ADG than COM, which was not different from LMR. This could be due to increased feed wastage of PMR resulting in an over-estimation of feed intake.

Following weaning, pigs provided NO consumed feed 5.3 hours sooner than COM-fed pigs, but no differences were observed in feed intake directly following weaning. It was expected that creep feed would assist in the transition to consuming pelleted feed; however, the inverse was observed. It is possible that those not provided creep feed consumed feed sooner since those provided COM were likely satiated and/or the novelty of feed presence could have enticed earlier consumption for the pigs that did not have access to creep feed prior to weaning. Additionally, since litters were not mixed at weaning this could have limited stress associated with weaning and allowed an earlier feed intake compared to what is often observed in commercial scenarios allowing satiation to drive feed intake. Overall, during the nursery period, creep feed treatment had limited effects on growth performance, only showing a tendency that LMR pigs had greater body weight compared to NO pigs at the end of the nursery period.

On the other hand, nursery diet complexity was a major influencer of growth performance during the nursery period. In general, those fed the high-complexity nursery diet had greater BW, ADG, ADFI and G:F. This improvement in nursery performance attributed to the high-complexity diet has been observed in previous studies (Skinner et al., 2014). However, others have shown that the pigs provided the low-complexity diet could catch up through the grower/finisher phase (Skinner et al., 2014). The current study did not continue beyond the nursery phase, but it is possible that a similar result could have been observed for these pigs. It was expected that creep feeding would improve the growth of pigs provided a LOW-complexity nursery diet post-weaning, however this was not the case as there was limited interactive effects between creep feeding and nursery dietary treatment on nursery growth performance. Furthermore, the provision of a more expensive creep feed (i.e. LMR) did not translate into better utilization of the low-complexity diet.

Therefore, nursery diet composition had a greater impact on nursery growth performance than the provision of creep feed, regardless of creep feed composition or form.

At the end of the nursery period, PMR- and LOW-fed pigs had greater relative weights of visceral organs (i.e. full gut weight, emptied stomach, small intestine, large intestine and liver). Visceral organ development after weaning is vital for nutrient absorption. Despite these differences observed for organ weights, apparent nutrient digestibility (AID and ATTD) was not improved for PMR- or LOW-fed pigs. It has been reported that pigs provided diets that are less digestible develop visceral organs with greater mass to compensate for the less digestible diet (Jones et al., 2012; English et al., 1980; cited by Pluske, 2016), which could explain the results of the current study. For example, pigs provided the low-complexity diet required greater surface area to break down and absorb nutrients in order to achieve AID and ATTD similar to pigs provided a high-complexity diet. During phase I, pigs that received the HIGH nursery diet tended to have greater OM AID than pigs that received the LOW nursery diet, but the latter adapted to the low-complexity diet by the end of the nursery period.

Following weaning, pigs provided COM during the suckling period had a longer fasting interval than those not provided creep feed, experienced a reduction in mucosal specific enzyme activity of maltase, and had reduced jejunal absorptive capacity versus pigs that did not receive creep feed. However, ileal absorptive capacity for COM-fed pigs tended to be greater than those fed NO when provided a low-complexity diet. Therefore, when piglets are provided creep feed, they are better able to cope with a low-complexity diet than those not provided creep feed. Furthermore, pigs provided PMR tended to have higher maltase specific activity than those provided COM on day 28, but by the end of the nursery PMR pigs had heavier relative organ weights indicating that a greater proportion of dietary energy was being partitioned toward protein deposition within the viscera and subsequently, maintenance requirements. Although those provided COM seemed the most affected by weaning in terms of reducing enzyme activities and absorptive capacity, they were not impaired at the end of the nursery period in terms of immune response, AID or ATTD.

Although pigs provided the LMR had the greatest BW at weaning, there were limited carry-over benefits in the nursery period. Furthermore, providing LMR was the most expensive creep feeding option, overall costing about $2.00 per pig more than the other creep feed treatments throughout the creep feeding period. Additionally, those fed LMR cost more per kg of final nursery BW than the other creep feed treatments (including NO), therefore showing that the increased feed cost did not translate to a proportional increase in growth. The high-complexity diet also cost about $4.30 more per pig over the nursery period than the LOW diet and is more expensive (cost/kg of exit BW) but the improvement in BW, visceral organ weight and gut morphology makes the HIGH-complexity diet advantageous. At the end of the nursery period those provided the high-complexity diet weighed more than the creep feeding counterparts provided the low-complexity diet, apart from those provided PMR. Therefore, by the end of the nursery period those provided LMR-HIGH and COM-HIGH were the heaviest. Although there was no significant interaction in cost of feed per kg of exit BW between creep and nursery treatments, those provided COM had the lowest cost/kg of exit BW basis of all treatments provided the high-complexity nursery diet.

Finally, this thesis showed that nursery diet complexity had a greater effect than creep feeding regimen on piglet growth during the nursery period. Although differences were observed for mucosal specific enzyme activities following weaning, it is possible that relatively longer feeding latency was driving the reduction in the specific enzyme activities between weaning and one-week post-weaning. Currently, total feed intake has been correlated to the regulation of enzyme activities, however this was not observed in this current study. More research should be done to determine if feeding latency is driving the change in enzyme activity. Additionally, the rate of recovery regarding specific mucosal enzyme activities is unknown for pigs past 7 days post-weaning. It is possible that although COM pigs experienced the greatest reduction in specific mucosal enzyme activities and gut morphology, they may have recovered during phase II of the nursery, whereas those provided a PMR were still compensating with larger visceral organs. By taking mucosal samples more frequently researchers would be able to determine the rate of recovery in terms of mucosal specific enzyme activities for those provided the different creep feed treatments.

In conclusion, piglets consumed greater quantities of liquid milk replacer during the suckling phase versus pelleted diets, and although this resulted in heavier pigs at weaning, it did not result in pigs that were better adapted to consuming a low-complexity diet in the nursery period. However, pigs provided a commercial creep feed (COM) had greater absorptive capacity than those not provided creep feed (NO) when given a low-complexity diet. However, this improvement in absorptive capacity for COM did not translate to improved growth for these pigs. Generally, pigs provided the high-complexity diet performed better (i.e. for ADG, ADFI, G:F, BW, VH:CD) than pigs provided the low-complexity diet, regardless of creep feed treatment. Therefore, a pelleted creep feed or no creep feed at all are viable pre-weaning feed management options, if pigs are provided a high-complexity diet after weaning.

4.8 REFERENCES

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APPENDIX A: LACTATION DIET

Item	Lactation Diet[1]
Ingredient composition, % (as-fed)	
Corn	48.28
Soybean meal	21.30
Barley	10.00
Wheat	12.40
Animal and vegetable fat blend	3.20
Limestone	1.65
Mono-calcium Phosphate	0.75
Sodium chloride	0.49
Lysine	0.43
Commercial micro premix	1.40
Threonine	0.10
Total	100
Calculated nutrient contents, as-fed	
Crude protein, %	17.00
Crude fat, %	5.47
Calcium, %	0.96
Phosphorus, %	0.55
NE, kcal/kg	2519

[1] Lactation diet provided from day 110 of gestation until weaning