# Crude protein content and probiotic supplementation in sows: effects on sow and piglet performance and indices of gut health

by

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# ABSTRACT

Since the use of in-feed antibiotics has been banned in many jurisdictions, alternative methods that maintain animal performance and health are being investigated. The objective of this study was to investigate the effects of crude protein (**CP**) content and supplementation of Bacillus-based probiotics (**PRO**) in late-gestation and lactation sow diets on performance of sows and gut health of suckling pigs. A total of 120 pregnant sows (60 gilts and 60 2+ parity) were assigned to one of four dietary treatments in a 2 x 2 factorial arrangement (2 CP levels, with or without PRO supplementation) from d 85 of gestation to d 21 of lactation. The experiment was conducted in 4 consecutive periods (half gilts and half sows per period). The diets consisted of 1) a corn-soybean meal (SBM)-based low CP (**LCP**; 12% CP) diet; 2) LCP + 0.05% probiotic (**LCP-PRO**); 3) a corn-SBM-based high CP (**HCP**; 15% CP) diet; and 4) HCP + 0.05% probiotic (**HCP-PRO**) and were formulated in 2 phases: gestation (d 85 – 115) and lactation (d 0 – 21). Commercially swine diets often contain approximately15% CP, therefore the HCP diet in this study reflects the industry standard. Lactation diets contained 14% and 17% CP for LCP and HCP respectively. Sow body weight (**BW**) and backfat (**BF**) thickness were recorded on d 85 and 111 of gestation and on d 1 and 21 of lactation. Sow blood and milk were sampled on d 1 and 17 of lactation to analyze plasma urea nitrogen (**PUN**) concentration, and milk fat, protein, and lactose, respectively. Reproductive performance data were recorded on d 0 of lactation and sow feces were collected d 12 of lactation for digestibility and microbial analysis. One piglet per sow close to average BW for each litter was selected for blood collection to analyze PUN and fecal collection for microbial analysis. Data were analyzed using the PROC MIXED of SAS. The statistical model included CP, PRO and their interaction as fixed effects, and period and replication as random effects. There was an interaction ($P < 0.05$) between CP and PRO on the number of stillbirths, where the number of stillbirths did not differ between LCP and LCP-PRO groups, while the number decreased in sows fed the HCP-PRO diet compared with the HCP diet. Sows fed LCP diets had reduced ($P < 0.05$) PUN concentrations on d 1 and d 17 of lactation compared with those fed HCP diets. No difference was found in fat, protein, or lactose content in both colostrum (d 1) and milk (d 17). Piglets from the sows fed LCP diets showed heavier ($P < 0.05$) birth weights (1.58, 1.57, 1.53, and 1.52 kg for diets 1 to 4) than those in HCP groups, however, the average daily gain (ADG) of week 1 (188, 166, 187, and 187 g for diets 1 to 4) was lower ($P < 0.05$) for piglets in LCP groups than HCP groups. There was an interaction ($P < 0.05$) between CP and PRO on piglet fecal DM, where HCP-PRO had higher fecal DM percentage than HCP, however, LCP and LCP-PRO groups did not differ. There was an interaction of CP and PRO on d 21 relative abundance of *E. coli*, where the abundance of was lower ($P < 0.05$) in piglets from sows fed HCP-PRO diets compared to piglets from sows fed HCP diets, however, piglets from sows fed LCP diet had a lower abundance ($P < 0.05$) of *E.coli* than piglets from sows receiving LCP-PRO diets. There was an interaction effect of CP and PRO on the relative abundance of *Lactobacillus* in piglets at age 21. Piglets from sows receiving HCP diets had a lower ($P < 0.05$) relative abundance compared to piglets from sows receiving HCP-PRO diets. Conversely, piglets from sows fed LCP-PRO diets had a lower ($P < 0.05$) relative abundance of *Lactobacillus* compared to piglets from sows fed LCP diets. On d 28, piglets from sows fed LCP diets had a decreased ($P < 0.05$) relative abundance of *E.coli* compared to pigs from sows fed HCP diets. There was an interaction effect of CP and PRO on d 28 where piglets from sows fed HCP diets had a decreased ($P < 0.05$) relative abundance of *Clostridium perfringens* compared to those from sows fed HCP-PRO diets. In conclusion, feeding LCP diets supplemented with PRO

to sows has the potential to improve both the reproductive performance of sows and the gut health of suckling pigs.

# DEDICATION

I would like to dedicate this thesis to my mother Leslie Chrapun and my brother Cory Chrapun for helping to keep me sane throughout my undergraduate and M.Sc. degrees. I will never be able to express how much I appreciate you both for sticking by my side and giving me the motivation to keep going. I would also like to dedicate this thesis to my late Baba Bette Shemeluk for pushing me to keep up with my studies through many challenging times.

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# FOREWARD

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# AUTHOR CONTRIBUTIONS

**Leah E. Chrapun**: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing–original draft.

**Jinyoung Lee**: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing–review & editing

**John K. Htoo**: conceptualization, methodology, writing–review & editing

**S. Maria Mendoza**: conceptualization, methodology, writing–review & editing

**Charles Martin Nyachoti**: conceptualization, supervision, project administration, writing–review & editing

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| | |
|---|---|
| **AA** | Amino acids |
| **ADFI** | Average daily feed intake |
| **ADG** | Average daily gain |
| **BF** | Backfat thickness |
| **BW** | Body weight |
| **CAA** | Crystalline amino acids |
| **CD** | Crypt depth |
| **CP** | Crude protein |
| **DM** | Dry matter |
| **EAA** | Essential amino acid(s) |
| **ETEC** | Enterotoxigenic *Escherichia coli* |
| **GIT** | Gastrointestinal tract |
| **HCP** | High crude protein |
| **LCP** | Low crude protein |
| **MAMP** | Microbe-associated molecular patterns |
| **PRO** | Probiotic(s) |
| **PRR** | Pattern recognition receptors |
| **PUN** | Plasma urea nitrogen |
| **PWD** | Post-weaning diarrhea |

| | |
|---|---|
| **SCFA** | Short-chain fatty acids |
| **SI** | Small intestine |
| **VCR** | Villus-crypt ratio |
| **VH** | Villus height |

# CHAPTER 1: GENERAL INTRODUCTION

Achieving high production efficiency is crucial for swine at every stage of production. In previous years, antibiotics were administered to pigs at different stages of production to promote growth and production efficiency. In addition to achieving high production potential, the use of in-feed antibiotics reduced the incidence of illnesses, such as post-weaning diarrhea (**PWD**) by balancing microbial populations in the gut. In the present day, the use of in-feed antibiotics is banned in Canada and many other countries due to growing concerns about antimicrobial resistant bacteria, therefore, alternative measures to maintain high productivity and microbial balance of the gut are being investigated.

The performance of the sow is crucial to overall herd productivity as it directly impacts the number of piglets born and subsequently weaned, which are key determinants of the farms' output and profitability. Antibiotics increased reproductive performance of sows by increasing litter size, although little is known about why this outcome occurred. There have been a variety of strategies tested to improve sow performance. Some of these strategies include phytogenic preparations such as essential oils and plant extracts, gonadotrophins, and yeast compounds (Santos et al., 2023; Hidalgo et al., 2014; Nguyen et al., 2016). These methods have been tested in the past, leading to improvements in reproductive performance such as increases in the number of piglets born alive (Reyes-Camacho et al., 2020).

Another method to maintain sow performance is reductions in dietary crude protein (**CP**). This may be the most cost-effective method to maintain performance as protein ingredients are the most expensive components of the diet; therefore, low crude protein diets may not only improve the reproductive performance of sows but may also lead to reduced feed cost. Many physiological and metabolic functions in the sow such as mammary development, placental growth, and maintaining body condition during pregnancy are influenced by dietary CP level (Kim et al., 2023). Providing approximately 15% CP (NRC, 2012) in the sow diet is important in improving protein utilization and deposition to ensure the animal maintains its productivity. Although CP content of the diet is important in sow performance, there is also evidence that reducing dietary CP can improve reproductive performance. It was reported that sows receiving LCP diets gave birth to more total piglets and more piglets born alive than sows receiving HCP diets. Additionally, LCP diets improved the quality of colostrum by increasing casein, protein, total solids, and non-fat solids in the colostrum and decreased the number of piglet deaths at weaning compared to sows receiving HCP diets (Fang et al., 2019). Moreover, the growth of suckling pigs is heavily influenced by the diet of the sow, as nutrients are passed from sow to piglet via milk and colostrum. Reduction in sow dietary CP level can reduce growth in suckling pigs due to inhibition of digestive enzymes and retardation of villus morphology, however, feeding sows LCP diets can reduce the number of harmful bacteria in the gut of suckling pigs (Kim et al., 2023). Piglets receiving milk from a sow fed a diet containing the standard amount of protein (~15% CP) can lead to a higher incidence of PWD since high amounts of undigested protein can allow proliferation of pathogenic bacteria in the sow, which can be passed to the piglet through colostrum (Marchetti et al., 2023).

A popular method currently being investigated to improve sow performance and piglet performance and gut health are supplementing PRO into the diet. Traditionally, PRO are provided in conjunction with LCP diets to fight illnesses by providing energy to help the gut fight off pathogens (Davila et al., 2013). They can do this by increasing digestion efficiency and producing antioxidants to protect healthy cells, which support growth and activity of immune cells and produce antibodies to fight infection (Nowland et al., 2019; Davila et al., 2013). For sow reproductive performance, PRO have been shown to increase litter size, litter weights and sow feed consumption during the lactation period (Böhmer et al., 2006; Hayakawa et al., 2016). Hayakawa et al., (2016) also reported that supplementing Bacillus-strain PRO resulted in an improved return to estrus ratio compared to control sows. Gut health is a major factor that contributes to overall efficiency in both sows and suckling pigs. Gut health can be improved by PRO supplementation by regulating intestinal flora and improving intestinal mucosa and barrier function, which can aid in preventing illness and increase growth performance in weaned pigs (Meng et al., 2010). There have been reports the sows receiving dietary PRO and piglets from sows receiving dietary PRO had longer villi in the ileum, resulting in thicker mucosa (Konieczka et al., 2023). This in turn improved the growth performance of the piglets as digestion and absorption of nutrients are greater when villi in the SI are longer (Szabó et al., 2023).

Many alternatives to antibiotics have been tested to maintain the production of weaned pigs. Although there are various studies investigating the effects of LCP diets and PRO separately in weaned pig diets, there is little literature into the synergistic effects of LCP diets and PRO, specifically on performance of sows, and performance and gut health of suckling pigs. For this reason, the current study aims to investigate the conjoint effects of LCP diets and PRO supplementation in the sow diet on performance of sows, and performance and gut health of suckling pigs.

# CHAPTER 2: LITERATURE REVIEW

## 2.1. INTRODUCTION

Antibiotics have been used in swine production for many years. They have been used to promote growth and can aid in reducing harmful bacterial growth in the gut, allowing the animal to increase nutrient absorption, and therefore increase growth performance (Butaye et al., 2003). Since antibiotics can reduce the presence of harmful bacteria in the gut, this can improve the intestinal health of the animal, leading to reductions in illness and disease outbreak (Butaye et al., 2003). The constant use of antibiotics has led to mass antibiotic resistance in swine populations, which makes it difficult to control disease if outbreaks occur (Butaye et al., 2003). Since the use of in-feed antibiotics has been banned in many jurisdictions, alternative strategies are being investigated to maintain high performance of pigs. One strategy is a reduction in dietary CP content coupled with crystalline amino acid (CAA) supplementation. Many aspects of sow performance rely on CP content. It aids in mammary development, placental growth, and maintenance during the gestation and lactation stages of production (Nowland et al., 2019). The lactation diet needs to contain enough CP for the sow as a significant amount of energy and protein is required for milk production. If this energy is not supplied in the diet, the sow may lose body condition and produce less milk, which can lead to smaller piglets at weaning and a longer wean to estrus interval (Huber et al., 2015). The amount of CP in the sow diet is also important for piglets, as providing enough CP to the sow can lead to increased growth performance in piglets by increasing average daily gain (ADG) and average daily feed intake (ADFI) (Lin et al., 2018). The level of CP in the sow diet is also important in nursery pig production as it supplies the pig with milk high in immunoglobulins and protein, which allows the pig to fight infections such as PWD. Pigs moving into the nursery are exposed to new stressors such as changes in diet, the environment, and new social structures which can cause issues such as oxidative stress and inflammation (Rhouma et al., 2017). These issues in conjunction with an immature immune system can make pigs susceptible to PWD, which is an enteric disease that can cause lethargy, gastric ulceration and severe diarrhea which can lead to death (Rhouma et al., 2017). There is evidence that LCP diets fed to weaned pigs can decrease the growth performance because the animal is receiving less nutrients (Nyachoti et al., 2006), however, it has also been stated that piglets from sows receiving LCP diets can show reduced incidence of diarrhea (Wang et al., 2022).

Another strategy being investigated to maintain high production in swine without antibiotic use is dietary supplementation with PRO. Probiotic supplementation in pig diets is often done in conjunction with feeding LCP diets to aid in fighting infection by providing energy and helping the gut fight off pathogens. There is evidence that PRO have the potential to provide more energy by being more efficient in digestion and can be beneficial in producing antioxidants to protect healthy cells, which support growth and activity of immune cells, and produce antibodies to fight infection (Nowland et al., 2019; Davila et al., 2013). There are many studies that depict beneficial results of PRO supplementation in the sow diet on weaned pig performance. Studies state that supplementing PRO to sows has the potential to improve feed efficiency, gut health and alleviate weaning stress in piglets (Luise et al., 2021). There is evidence that PRO can improve gut health by regulating intestinal flora balance, improving intestinal mucosa, and improving intestinal barrier function, which in turn can aid in preventing illness and can increase growth performance (Luise et al., 2021). There is a gap in the literature on the potential synergistic effects of LCP and PRO on sow performance. The objective of this review is to provide an overview of current knowledge regarding the use of LCP diets and PRO supplementation on performance of sows and their progeny.

## 2.2. POST-WEANING DIARRHEA

Post-weaning diarrhea (PWD) is an enteric disease that occurs in the first 2 weeks post-weaning in piglets and involves interactions between the sow, the piglet, the environment, enterotoxigenic *Escherichia coli* (ETEC) bacteria and livestock management (Rhouma et al., 2017). In swine production, PWD is one of the most prevalent and harmful illnesses which cause massive losses in production. Infection of PWD begins with adhesion of ETEC bacteria to the intestinal microvilli via host-specific fimbriae (Wang and Gänzle, 2019) . In swine, the fimbriae that mediate adhesion to intestinal mucosa and can cause diarrhea-related illness are F18 and K88 (Fairbrother et al., 2005). The fimbriae attach to the intestinal brush border and activate the secretion of enterotoxins which can cause severe diarrhea by disturbing the secretion and absorption of water by enterocytes (Rhouma et al., 2017). The immature gut microbiome in conjunction with high stress levels create the optimal environment for *E.coli* to survive and proliferate. The symptoms of PWD include severe diarrhea, which can cause reduced feed intake and digestibility, growth retardation, lethargy, dehydration, anorexia, and death (Rhouma et al., 2017). In the past, PWD has been treated with antibiotics, however due to antimicrobial resistance, methods to prevent adhesion of *E.coli* to the intestinal wall are being investigated, such as the use of LCP diets supplemented with CAA and PRO supplementation in sow diets.

## 2.3. WEANING STRESS

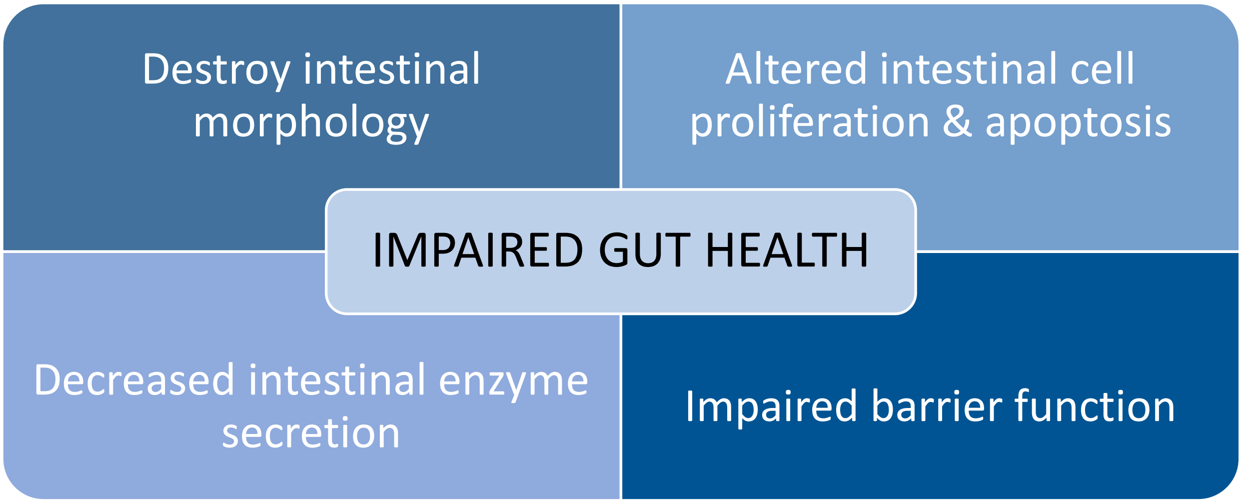
Weaning is a very stressful event for piglets. Piglets are typically weaned at approximately nineteen to twenty-two days of age (Holman et al., 2021), whereas wild pigs are normally weaned from their mother at the age of four months (Postma, 2019). Conventional pig production allows for a short period of time the piglet can stay with its mother, and the subsequent move to the nursery can cause separation stress. When pigs enter the nursery, they are fed solid feeds that are less digestible than the milk they have been receiving (Holman et al., 2021). Since this feed is less digestible, it can cause reductions in growth, which can lead to reductions in overall performance of these animals. These are a few of the many stressors pigs face when entering the nursery, and these stressors can impair the function of the GIT.

### 2.3.1. CHANGES IN PIGLET GUT STRUCTURE AND FUNCTION

The small intestine (SI) is the main site of nutrient absorption in swine, and it helps to prevent circulation of pathogenic bacteria and toxins (Tang et al., 2022b). There are four main changes in weaned pigs' GIT that can cause detrimental effects, which are depicted in Figure 2.3. After weaning, the GIT changes significantly and may cause damages in intestinal morphology. Villus height (VH), crypt depth (CD), and the villus to crypt depth ratio (VCR) reflect the health of the intestinal tract (Tang et al., 2022a). When VH and VCR are reduced, intestinal mucosal function is impaired which reduces nutrient absorption. This is more likely to occur during weaning because stress can inflate these issues.

There is substantial evidence that shows that weaned pigs have decreased VH and VCR after weaning compared to before weaning. Bomba et al. (2014) saw decreased VH and VCR in pigs five days post-weaning compared to pre-weaning. In addition, Hu et al. (2013) reported

**Figure 2.3.** Detrimental effects in weaned pig gut health



(Nowland et al., 2019)

degradation of gut morphology in which VH and VCR were reduced in pigs on day three and seven post-weaning, and it did not return to pre-weaning levels until day fourteen post weaning. Another article by Boudry et al. (2004) also reported decreased VH and VCR in weaned pigs that lasted until day fifteen post-weaning. Prior to weaning, the sows provide adequate nutrition to the piglets through colostrum and milk which increases the activity of digestive enzymes such as lactase, protease, and lipase (St-Pierre et al., 2023). Due to changes in diet composition and reduced feed intake, the activity of enzymes decreased once the piglet is weaned, causing reduction in digestion and absorption of nutrients (Boudry et al., 2007; Xiong et al., 2019). Since weaning can cause high stress and the pigs are no longer receiving these enzymes to break down feed, their feed intake may decrease which can cause reductions in VH and VCR (Tang et al., 2022).

Weaning stress has also been shown to alter the amount of intestinal epithelial cells, cell proliferation and apoptosis. The intestines are tissues that are self-renewing, and the integrity of the SI require harmony between the amount of cell proliferation and cell apoptosis, also known as cell death (Tang et al., 2022). The production of intestinal epithelial cells is important in proliferation of crypt stem cells (Xiong et al., 2016). Crypt stem cells play a role in generating fast developing cells with specific functions such as absorptive epithelial cells (Liu et al., 2020). Apoptosis is important in growth and development of the GIT, however due to weaning stress, the rate at which cells are generated and dying are obstructed. This increases the intestinal mucosal permeability and affects barrier function, which can lead to harmful bacteria attaching to the SI lining, which can cause severe illness in weaned pigs (Tang et al., 2022). Yang et al. (2016), and Wang et al. (2008) reported that the expression of genes related to cell proliferation are increased in weaned pigs, and Zhu et al. (2014), Tang et al. (2021), and Cai et al. (2016)

reported that the expression of genes related to cell apoptosis are increased in jejunal cells of weaned pigs. This can be explained by an article by Yang et al. (2016), which states that since weaned pigs are stressed, they reduce their feed intake leading to malnutrition. Malnutrition of weaned pigs can cause issues in energy and protein metabolism which can negatively affect proliferation of intestinal crypt cells, leading to unbalanced cell proliferation and apoptosis.

As previously mentioned, the activity of digestive enzymes decreases after weaning due to decreased feed intake and a change of diet. Weaned pigs have the ability to secrete intestinal enzymes to breakdown nutrients, however they will not be produced in optimal quantities as weaned pigs do not have a fully developed GIT (Zhu et al., 2015). Weaning stress can also decrease intestinal enzyme secretion which can leave the pigs more susceptible to malnutrition and disease. The enzymes required to breakdown carbohydrates, such as lactase, maltase, and sucrase, are some of the most important enzymes inhibited because if they are not secreted in sufficient quantities, it can cause severe diarrhea in weaned pigs, which is likely to cause death.

Intestinal barrier function is important for swine health. The intestinal barrier prevents pathogens from attaching to the SI surface, ensuring optimal digestion and absorption of nutrients to optimize production performance (Tang et al., 2022b). Weaning stress can cause negative effects of barrier function which can cause intestinal inflammation and increase permeability. The components of the barrier include intestinal epithelial cells, an intestinal mucus layer, immune cells, and healthy microbes, and they are divided into 4 groups including the mechanical (physical), chemical, immune, and microbial barriers (Gou et al., 2022; Tang et al., 2022b, Xu et al., 2022). The microbial and immune barriers will be discussed later in this review (Section 2.3.2 and 2.3.3 respectively).

The mechanical intestinal barrier is composed of a single layer of epithelial cells which are linked by tight junctions and adherens junctions. These junctions reduce the area between cells which increases absorption of nutrients into the body and decreases the ability for pathogens to attach to the intestinal surface (Gou et al., 2022; Tang et al., 2022b). Weaning stress can cause breakdown of junctions which can increase the permeability of the SI, causing pathogens to break the mucosal barrier and enter other tissues via the circulatory system. In contrast, the chemical barrier is composed of a mucous layer that contains mucins produced by goblet cells and antimicrobial proteins produced by epithelial cells (Xu et al., 2022). The chemical barrier acts as the "first line of defense" for intestinal epithelial cells from toxins or harmful bacteria (Tang et al., 2022b). If maintained, it aids in preventing the colonization of harmful bacteria in the GIT, leading to increased feed efficiency, however, weaning can cause decreased mucin production which causes the mucin layer to become thin, making it easier for pathogens to permeate the barrier (Moeser et al., 2007; Smith et al., 2010). After pathogens have broken the barrier, they compete with healthy bacteria to attach to the surface of the intestinal mucosa, damaging the intestinal barrier. Once attached, they activate apoptosis of epithelial cells and disrupt production of tight junction proteins between mucosal cells (Hu et al., 2013; Moeser et al., 2007). All of this causes inflammation and damage to the intestinal barrier, making the pig more susceptible to disease. Maintaining the integrity of the mechanical and chemical intestinal barriers is crucial to prevent illness, such as PWD, which can cause significant losses in the production system.

### 2.3.2. CHANGES IN PIGLET GUT MICROBIAL COMMUNITIES

The microbial structure of a weaned pigs' GIT is very intricate and goes through dynamic transformations. As piglets transition from sow milk to solid feed, many changes are made in the microbial communities of the gut, which shape their digestive health and productivity. Piglets receiving solid feed post-weaning cannot fully digest and utilize all nutrients provided because they have an immature digestive system, which impedes complete digestion of nutrients (Chen et al., 2018). Diarrhea is a common symptom of weaning stress, which can also lead to changes in the intestinal microbiota of piglets. The microbiota in the gut is responsible for digestion and absorption of nutrients. If the microbiota in the gut is unbalanced, this can cause intestinal cell apoptosis, mechanical barrier damage and deterioration of intestinal immune function (Chen et al., 2018). Colonization of gut microbiota occurs at birth as the piglet is introduced to new bacteria from both the sow and the environment. The colonization is influenced by consumption of colostrum and milk. When piglets are weaned, they are no longer consuming beneficial bacteria provided from the sow, which can cause an imbalance between healthy and pathogenic bacteria in the GIT, causing a decrease in feed efficiency and production potential. Arguello et al. (2018) reported an increase in pathogenic bacteria (Citrobacter) and a decrease in beneficial bacteria such as *Bifidobacterium* and *Lactobacillus* in the ileum mucosa of weaned pigs. Sun et al. (2019), and Yang et al. (2019) found that weaned pigs suffering from diarrhea had a lower relative abundance of Bacteroides, which are healthy bacterium that provide protection from pathogens and supply nutrients to other gut microbes (Zafar & Saier Jr., 2021). Zafar and Saier Jr. (2021) also reported lower relative abundances of Ruminococcus, Bulleidia, and Treponema in weaned pigs suffering from diarrhea, which are also healthy bacteria that aid in the digestion of nutrients. To summarize, weaning can cause imbalances between healthy and pathogenic bacteria because they are no longer consuming beneficial bacteria from sow milk and are exposed to new harmful pathogens from the environment and other pigs.

### 2.3.3. CHANGES IN PIGLET IMMUNE FUNCTION

Sow milk contains many beneficial elements for the piglets. It contains immunoglobulins, and T- and B-cells, which allows the piglet to better fight off pathogens, reducing susceptibility to severe illness (Curtis and Bourne, 1971). Weaning can have a massive impact on the immune function of pigs. Immune function of pigs does not fully develop until seven weeks of age (Stokes et al., 2004). Pigs are weaned at approximately four weeks old, therefore they are not fully capable of fighting off pathogens the same way adult pigs can, leading to increased susceptibility to illness. The intestinal immune barrier is a complex system consisting of immune cells, such as lymphocytes, neutrophils and macrophages, immune molecules, such as antibacterial peptides and immunoglobulins, and immune organs (Tang et al., 2022a,b). The immune barrier generates immune response to pathogens and builds immune tolerance to common antigens in the environment. If working properly, intestinal epithelial cells recognize pathogenic substances and beneficial bacteria through pattern recognition receptors (PRR) such as toll-like receptors, which aid in development of adaptive immunity (Tang et al., 2022a). At weaning, pathogens such as *Escherichia coli* and *Salmonella spp.* are present in high quantities, which can lead to inflammation in the gut. This can lead to illness such as PWD and can cause serious damage to the GIT of weaned pigs. Weaning stress can cause the intestinal barrier to become more permeable, leading to pathogens breaking the barrier and entering systemic tissues. Spreeuwenberg et al. (2001) reported that the $CD4^+/CD8^+$ T-lymphocyte (T-cell) ratio was decreased after weaning, which is indicative of lower immune function. Pietrasina et al. (2020)

also reported decreases in B-lymphocytes (CD21$^+$; B-cell) at weaning, which decrease immune response to pathogenic bacteria. Although T-cell activation is important in fighting illness, activation can cause an increase in the secretion of proinflammatory cytokines, which has been shown to decrease motivation to socialize and feed in weaned pigs (Nordgreen et al., 2020). If pigs experience this, it may cause them to reduce feed intake, which can cause issues such as lethargy and starvation.

## 2.4. METHODS TO PREVENT POST-WEANING DIARRHEA

Preventing PWD is crucial for the health and well-being of pigs. This condition, often triggered by abrupt dietary changes and stress during the transition from sow's milk to solid feed, not only compromises piglet health but also poses significant economic challenges for farmers (Rhouma et al., 2017). This illness can lead to reduced growth rates, increased mortality rates, and higher veterinary costs, impacting overall productivity and profitability (Rhouma et al., 2017). Therefore, implementing effective strategies to mitigate PWD is essential to ensure optimal growth, minimize economic losses, and maintain the welfare standards of piglets in intensive farming systems.

There are many methods that were commonly used to prevent PWD in nursery pigs. Antibiotics were administered to pigs at subtherapeutic levels through the feed to increase growth and prevent the presence of pathogenic bacteria in the gut that could cause illnesses such as PWD. Antibiotics were often provided in conjunction with high amounts of trace minerals such as zinc oxide and copper sulfate. Zinc oxide has many positive effects on pigs such as improving the activity of enzymes involved in digestion, cellular respiration, and nucleic acid metabolism (Hill & Shannon, 2019). Although the mode of action of zinc oxide to prevent PWD is not well understood, the general consensus it that it improves nutrient absorption and intestinal morphology, improving the intestinal barrier function, reducing permeability, and thus preventing adhesion and proliferation of PWD-causing bacteria such as *E. coli*. The proposed mode of action of zinc oxide is shown in table 2.3, which is adapted by Bonetti et al. (2021).

Copper sulfate is another trace mineral that has been given with antibiotics to investigate the effects in preventing PWD. Similar to zinc oxide, it has antimicrobial properties which can prevent adhesion of PWD-causing bacteria (Bonetti et al., 2021). It can also improve gut health by improving the growth of beneficial bacteria, and can strengthens the intestinal barrier to reduce permeability, decreasing the probability of infection (Bonetti et al., 2021). While both these elements have beneficial effects on the host, they have been shown to have negative environmental impacts. The presence of zinc oxide and copper sulfate in the environment has led to contaminated waterways causing disruption of aquatic ecosystems. In addition, many producers use manure as fertilizer. Pigs receiving zinc or copper as a means to prevent PWD will excrete these elements in their feces, therefore if manure is used on fields, it can lead to accumulation of these minerals in the soil which can affect soil health and fertility (Bonetti et al., 2021). Moreover, in-feed antibiotics have been banned globally due to microbial resistance, thus other methods to prevent PWD are being investigated.

Another method explored to prevent PWD is prebiotic supplementation. Prebiotics are bioactive compounds consisting of mostly fiber that resist digestion by enzymes in the GIT but are used as a substrate for beneficial bacteria in the gut which aids in promoting gut health (Kiernan et al., 2023). Prebiotics are considered forms of dietary fibre and dietary fibre has been said to reduce intestinal permeability, reducing the number of pathogenic bacteria in the gut that could lead to disease (Luo et al., 2022). Fibre can also act as a substrate for microbial fermentation in the gut short-chain fatty acids (SCFA). The SCFA produced by this process can be used as an energy source, which can be used for fighting infection (Fleming, & Rodriguez, 1983). There is evidence that SCFA can be beneficial to the gut by decreasing pH levels in the gut and increasing the diversity of microbiota of the gut, leading to better digestion and absorption of nutrients that can be used for energy to prevent and fight pathogens (Walker et al., 2005). Prebiotics are still being used as a way to prevent PWD in pigs, however, low CP diets and PRO supplementation into the sow diet have been investigated recently as they have also been shown to improve gut health and nutrient absorption, which can potentially reduce the risk of PWD in their progeny.

**Table 2.3.** Assumed mode of action of zinc oxide in preventing post-weaning diarrhea infection adapted from Bonetti et al. (2021)

| | |
|---|---|
| Intestinal morphology | Increase villi : crypt ratio<br>Increase tight junction expression |
| Digestion | Increase nutrient digestibility<br>Increase digestive enzyme activity<br>Increase nutrient absorption |
| Immune system | Decrease pro-inflammatory cytokines<br>Increase anti-inflammatory cytokines<br>Increase numbers of regulatory T-cells<br>Increase stem cell factor |
| Antimicrobial | Moderate antimicrobial effect<br>Decrease in bacterial adhesion to cells<br>Increase oxidative stress |
| Antioxidant | Increase intestinal sphincter of Oddi expression<br>Increase intestinal metallothionein |
| Secretion | Increase ghrelin secretion<br>Increase pancreatic enzyme secretion |

## 2.5. DIGESTION OF PROTEIN IN SWINE

Digestion of dietary protein is very important in supplying pigs with the required nutrients. Nutrients such as essential fatty acids, zinc and iron, and fibre allows pigs to reach their full production potential. In swine, protein digestion starts in the stomach, where gastric proteases and hydrochloric acid start the chemical digestion of protein, which denatures it into large peptides (Sitrin, 2014). Pepsinogen is released and hydrochloric acid activates it into pepsin (Sitrin, 2014). After the degradation in the stomach, the peptides descend into the small intestine. Pancreatic proteases are secreted as inactive proenzymes, such as trypsinogen, chymotrypsinogen, prolastase, and procarboxypeptidases (Yen, 2001). Enterokinase, which is secreted from the duodenal brush border, activates trypsinogen to trypsin, which activates other pancreatic proenzymes, which in turn break down large peptides into smaller peptides or amino acids (AA) in the small intestine (Yen, 2001). The last part of digestion in the small intestine involves the brush border peptidase and cytosolic peptidase. The brush border peptidase hydrolyzes peptides containing three or more AA, and the brush border peptidase and cytosolic peptidase work together to break down peptides smaller than three AA into free AA (Yen, 2001).

## 2.6. CRYSTALLINE AMINO ACID SUPPLEMENTATION IN LOW PROTEIN DIETS

There is a high environmental impact when feeding HCP diets to swine. Agriculture is the largest contributor to greenhouse gas emission, in which pork production contributes to 9% of total greenhouse gas emissions (Yang et al., 2023). The largest contribution to this is from feed production in the amount of 42.6% (FAO, 2022; MacLeod et al., 2013), and the second largest contributor to swine greenhouse gas emissions is manure (MacLeod et al., 2013). Urease enzyme converts the high amount of urea in the feces into ammonia, which deteriorates the soil and adds to air pollution (Lee et al., 2023). To reduce the environmental impact of swine production, LCP diets are being investigated. Lowering the CP content of the diet is thought to reduce growth performance because of the decreased availability of AA (Lee et al., 2023). Therefore, LCP diets are supplemented with CAA to meet the indispensable AA requirements for optimal performance. In contrast to intact protein sources, CAA are readily available and absorbed, whereas high protein sources such as soybean meal (SBM) are not completely broken down by digestive enzymes, thus they do not provide free AA that are easily absorbed by the body (Lue and Qiao, 2008). Therefore, feeding pigs LCP diets with CAA supplementation may increase the digestibility of CP, allowing for improved growth performance compared to pigs fed HCP diets. In addition, providing diets with LCP and CAA reduce the amount of undigested protein available for microbial fermentation which decreases the number of pathogenic bacteria in the gut, resulting in a lower risk of PWD and urea production (Wang et al., 2018). Lue and Qiao (2008) reported that fecal consistency of weaned pigs improved linearly as CP content decreased from 23.1% to 17.2%. In addition, Eugenio et al. (2022) fed diets with free AA to growing pigs and reported a higher VH and VCR in the jejunum compared to pigs fed diets with intact protein sources. Lee et al. (2023) reported that weaned pigs fed LCP diets with CAA supplementation had increased VH and VCR compared to pigs fed HCP diets. Increased VH directly affects the nutrient absorption capacity of the intestine because it increased the surface area for nutrient absorption (Chwen et al., 2013), therefore it has been shown that providing free form AA, such as CAA, can result in increased absorption capacity of the SI, leading to increased growth performance and decreased incidence of PWD.

There have been studies to shown that even with free form AA supplementation, LCP diets resulted in decreased growth performance of pigs. Spring et al. (2020) reported that feeding diets consisting of 15% CP with branched chain AA to weaned pigs resulted in decreased growth compared to pigs fed diets with 25% CP. Although Yue and Qiao (2008) reported an improvement in fecal consistency in pigs fed LCP diets with CAA supplementation, they also reported decreased growth performance, as well as decreased VH in the jejunum and duodenum in pigs fed 17.2% CP compared to pigs fed 23.1% CP. A decrease in VH indicates a decrease in absorptive capacity which can impact the production potential. Moreover, Peng et al. (2016) reported decreased growth, decreased VH and CD in duodenum, jejunum and ileum in weaned pigs fed LCP diets supplemented with indispensable AA compared to pigs fed HCP diets. Lee et al. (2023) reported that LCP diets supplemented with CAA fed to weaned pigs did not influence growth performance, but decreased feed efficiency compared to pigs fed HCP diets. Feed efficiency is a measure of how feed intake is converted into BW, therefore decreased feed efficiency indicates lower BW gain per amount of feed, which reduces production performance. Although there is extensive literature on the effects of LCP diet with CAA supplementation in weaned pigs, more research needs to be conducted to test the effects of LCP diets with CAA supplementation on sow and suckling pig performance and gut microbiome.

### 2.7. EFFECT OF CRUDE PROTEIN CONTENT OF THE SOW DIET ON SOW AND PIGLET PERFORMANCE

Similar to weaned pigs, sows can be subjected to many stressors such as multiple breeding services, changes in housing and feed, and weaning of piglets. This can cause shifts in the gut microbiome, which can reduce digestion of nutrients, leading to reductions in reproductive performance (Kim et al., 2021). For gestation sows, CP content of the diet should not be below 10-11% CP as severe reductions in CP content can negatively influence fetal development. For lactation sows, the CP content of the diet should not be lower than 13-14 CP. This is because if the sow is not consuming enough protein, it can negatively impact the growth performance of piglets and can decrease their chance of survival once weaned (Hojggard et al., 2020). Crude protein is important for sow mammary development, placental growth, and maintenance during gestation and lactation, so it was thought that reductions in sow dietary CP content could cause issues in performance. However, recently, several studies have suggested that feeding AA supplemented LCP diets maintain sow performance and a healthy gut and can have the same positive effects on reproductive performance as HCP diets. Kim et al. (2021) and Gregory et al. (2023) reported that sows receiving diets with 17% CP did not negatively affect piglet birth weights compared to sows receiving diets containing 19% CP. Mejia-Guadarrama et al. (2002) and Pike & Boaz. (2009) reported that feeding diets with 10% CP also did not negatively affect piglet initial BW or ADG of piglets compared to piglets from sows receiving diets with 20% and 19% CP respectively. Niyonsaba et al. (2023) and Han et al. (2023) saw a tendency for lower BW in pigs from sows fed LCP diets (14% and 20% CP respectively). These discrepancies could be explained by differences in parity. Segura et al. (2020) found that primiparous sows and sows of 4 or more parities had lower piglet BW than sows of two or three parity. In addition, Kim et al. (2021), Pike & Boaz. (2009), and Gregory et al. (2023) reported no difference in weaning weights of pigs from sows fed AA supplemented LCP diets compared to pigs from sows fed HCP diets, however, Hu et al. (2023) saw increases in weaning weights of piglets from sows fed 14.5% CP compared to piglets from sows fed 17.5% CP. The cause of this could be that LCP diets contained the optimal quantity of AA, as they were added in sufficient quantities in order to achieve an ideal protein ratio. The HCP diets may not have contained optimal AA ratios, which can reduce growth performance of these animals (Wang et al., 2018).

There have also been beneficial effects seen for LCP diets on total number of piglets born, piglets born alive, stillborn, and mummified. Gregory et al. (2023), Mejia-Guadarrama et al. (2002), and Corley et al. (1983) reported no differences in these reproductive criteria between sows receiving LCP (17%, 10%, and 12% CP respectively) and sows receiving HCP (19%, 20% and 16% CP respectively). Hu et al. (2023), Pike & Boaz. (2009), Pietzak & Grela. (2015), Luise et al. (2021), and Fang et al. (2019) state that sows receiving LCP diets had higher numbers of total born and liveborn piglets compared to sows receiving HCP diets. These findings can be explained by a study conducted by Pederson et al. (2019). The researchers state that excess protein in the diet can reduce feed efficiency because lots of energy is utilized in the synthesis of urea, which is excreted in the urine. Therefore, this energy cannot be used for piglet development during gestation, resulting in  lower numbers of total piglets born and piglets born alive.

Sow milk composition is very important for piglets. The immunoglobulins found in sow milk can provide increased immunity to the piglets which is crucial as active immunity, which is the immunity that results from the production of antibodies by the immune system to fight pathogens. Sow colostrum and milk also contains protein which is vital for the growth of piglets. The fat content of the milk is also important, as it has been shown to improve survivability of piglets and increase weaning weights (Azain, 1993). Studies have found that sows being fed LCP (17%, 14% & 12% CP respectively) diets had similar milk and colostrum composition compared to sows being fed HCP diets (19%, 19%, & 16% CP respectively) (Gregory et al., 2023; Kim et al., 2023; Corley et al., 1983). This can be possible as pigs receiving HCP diets have been shown to have increased milk protein content. Since the sow is receiving an abundance of protein, that protein can be used in many ways, such as sow maintenance and milk production (Kim et al., 2021). However, sows receiving LCP diets can digest and metabolize more of the protein provided as it takes less energy to breakdown the feed (Kroeske et al., 2021). The energy not used for feed breakdown can then be used for milk production, which can lead to similar results between sows receiving HCP and LCP diets (Kim et al., 2021). Fang et al. (2019) and Kim et al. (2023) reported that sows receiving LCP (~14% CP) diets have increased fat and protein content of milk compared to sows receiving HCP (~19% CP) diets. This may be possible as sows receiving LCP diets can easily digest the smaller amount of protein being provided, resulting in more protein being used in milk production (Kim et al., 2021).

## 2.8. EFFECT OF CRUDE PROTEIN CONTENT ON PIGLET AND WEANED PIG GUT HEALTH

Optimal CP inclusion in swine diets is important for many functions. For piglets, CP is very important for growth and development. During the early stages in life, the CP requirements of pigs are high because they are rapidly growing and developing various bodily systems. Insufficient CP intake can lead to growth retardation, weakened immune systems and various other health issues (Fang et al., 2019). After weaning, CP is important because it plays an important role in muscle deposition and growth performance. Diets with optimal levels of CP provide essential AA, which play a crucial role in muscle deposition (Fang et al., 2019). Among the AA, lysine is the first limiting AA which determines the amount of muscle deposition; therefore, enough lysine must be provided in the diet to maximize growth performance of weaned pigs. Although CP is important for growth performance, providing excess CP in the diet can cause other issues. Supplying excess CP in swine diets has been attributed to detrimental environmental impact (Fang et al., 2019). Pig production produces a significant amount of ammonia, which contributes to aerial pollution (Ball et al., 2022). In addition, due to runoff, manure that contains high amounts of nutrients can enter waterways which promotes growth of algae, depleting oxygen reserves in the water (Ball et al., 2022). When oxygen is depleted in the water, aquatic organisms will not receive enough oxygen which leads to death (Ball et al., 2022). Excess CP in the diet can cause issues for pigs at various stages of production, but especially piglets and weaned pigs. Since the GIT is not fully developed at this stage, it can be more difficult for the pig to digest and absorb all CP provided in the diet. This can lead to the presence of more non-digested substances which can be used for microbial fermentation. The survivability of pathogens in the GIT is increased when fermentable protein is present because the pathogens can use it for metabolism and energy sources (Kim et al., 2022). Harmful bacteria proliferation can then lead to illness such as PWD. To combat this, LCP diets are being investigated. Diets containing LCP are traditionally provided with AA supplementation in order to ensure the diet contains enough AA for bodily functions and growth. If diets with reduced CP are provided without addition of AA, piglet performance may be reduced as they will not possess enough essential AA in the body for growth (Kim et al., 2022).

There is evidence to show that LCP diets can have positive effects on piglet and weaned pig gut health, which is highly attributed to the supplementation of AA. As mentioned above, undigested protein is used for microbial metabolism via deamination (removal of amino group) and decarboxylation removal of carboxyl group) (Nogal et al., 2021). Carbohydrate fermentation is favoured in the gut compared to protein fermentation because the enzyme used to break down carbohydrates (decarboxylase) is active under acidic conditions and the large intestine also has acidic pH (Nogal et al., 2021). Carbohydrate fermentation has beneficial effects for the host, such SCFA production. The presence of SCFA in the body is important as they can improve gut barrier integrity, glucose, and lipid metabolism, and regulate the immune system and inflammatory response (Nogal et al., 2021). Microbial protein fermentation has detrimental effects for the animal. As previously mentioned, HCP diets can increase the amount of non-digested substrates, which provide energy sources for pathogenic bacteria to proliferate and colonize the gut, such as *E.coli*, which can cause a variety of effects including death. The endotoxins produced from ETEC, such as LT and ST enterotoxins, can change aspects of the GIT (Opapeju et al., 2015). This can cause decreased fluid absorption and increased frequency diarrhea. Various studies have reported that LCP diets can reduce ETEC numbers in the gut compared to pigs receiving HCP diets. Opapeju et al. (2015) reported that weaned pigs receiving a diet with 17.3% CP showed reduced ETEC counts in jejunal digesta (2.49±0.85 log10 CFU g−1) compared to pigs receiving a diet with 22.2% CP (3.65±1.44 log10 CFU g−1). These researchers also found that ETEC K88 colonies were found in 4 out of 5 pigs fed the HCP diets, and 1 of 5 pigs fed LCP diets. In later years, Opapeju et al. (2009) reported similar results. They showed that weaned pigs receiving LCP (17.6% CP) had less ETEC numbers in digesta of the ileum, rectum and colon compared to pigs fed the HCP (22.5% CP) diet on both day 3 and 7. Wellock et al. (2008) reported similar results, with numbers of fecal ETEC tending to be higher in weaned pigs receiving HCP (230g CP/kg) compared to LCP (130g CP/kg). Although CP content was not specified, Pollock et al. (2019) reported that ETEC content in ileal digesta samples of ETEC-exposed pigs receiving HCP diets was higher than in ETEC-exposed pigs receiving LCP diets. They also reported that ETEC-exposed pigs receiving HCP diet shed 10-fold more ETEC than ETEC-exposed pigs receiving LCP diet.

*Clostridium perfringens* is an anaerobic, Gram-positive bacteria that can cause issues in swine production. It can cause diarrhea, dysentery, and death in pigs if the infection persists (Rist et al., 2013). High CP intake can stimulate growth of many pathogenic bacteria such as

Clostridium. Fermentation of CP coincides with the growth of Clostridium because during this process, substrates are only partially broken down, leaving the remaining substate as a source of energy for pathogenic bacteria to thrive (Rist et al., 2013). Thus, *Clostridium perfringens* is of particular interest for piglets because of its ability to cause severe diarrhea. This illness affects pigs within the first 2 weeks of life and due to the piglets' weak immune systems, severe dehydration, weight loss and death are likely outcomes (Grześkowiak et al., 2018). In finishing pigs, Liao et al. (2024) found that reducing dietary CP in the diet led to reduced numbers of *Clostridium* bacteria in the gut. Wang et al. (2023) reported similar results, where pigs receiving diets containing 12% CP had reduced *Clostridium* counts than pigs receiving 14% and 16% CP. *Lactobacillus* spp. are also being investigated but for their inverse effects. It has been shown that *Lactobacillus* in the gut of pigs is beneficial as they aid in feed breakdown and suppress pathogenic bacteria (Dempsey and Corr, 2022). Reducing CP in the diet allows for reductions in pathogenic bacteria as this limits substrate availability and therefore the proliferation of pathogenic bacteria. With less pathogenic bacteria in the gut, beneficial bacteria such as *Lactobacillus spp*. will have less competition for energy sources, which will increase the abundance of beneficial bacteria in the gut microbiome. Gao et al. (2022) found that HCP diets led to decreased counts of *Lactobacillus* in the gut than LCP diets in weaned pigs. In addition, Mi et al. (2023) reported that weaned pigs receiving diets with 14% CP had higher numbers of *Lactobacillus* in the jejunum than pigs fed a diet containing 17% CP. Although there is significant research into the effects of CP content on gut health of weaned pigs, there is insufficient literature on the effects of CP in the sow diet on the gut health of suckling pigs, therefore, more research needs to be conducted to investigate these effects.

## 2.9. WHAT ARE PROBIOTICS?

The term “probiotic” is derived from the Greek/Latin word “pro” and the Greek word “bios” which signify “of life” (Moghaddam, 2011). The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) describe PRO as “live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host” (Bajagai et al., 2016; Iannitti and Palmieri, 2010). Products used as PRO can be either yeasts, bacterial cultures, or a mixture of the two that activate microbes in the GIT which can improve the gut microbiome, leading to improved gut health and growth performance (Liao and Nyachoti, 2017). Many commercial PRO used in livestock nutrition contain additional substances such as enzymes that can aid in feed breakdown and absorption of nutrients (Liao and Nyachoti, 2017). Commercial PRO can be characterized into 4 categories as seen in Table 2.9, adapted from Liao and Nyachoti (2017). The categories include single- or multi-species / strain, bacterial or non-bacterial, spore-forming, or non-spore-forming, and allochthonous or autochthonous.

Recently, there have been studies investigating the effects of PRO on animal production and health. Probiotics have been thought to be a good alternative to antibiotic use as they can balance the microbiome and prevent adhesion of pathogens to the GIT (Dominigos et al., 2021). Probiotics can resist breakdown by gastric acid and resist digestion in the upper digestive tract to interact with bacteria native to the host’s gut (Zhang et al., 2023). Although PRO are live bacterial organisms, they do not cause negative effects on the host such as illness (Zhang et al., 2023). The expected outcomes of PRO supplementation are shown in Figure 2.9. Probiotics can improve feed efficiency because they can balance the gut microbiota. The microbiota in the gut aid in degradation of feed that enzymes in the digestive tract cannot do. Beneficial bacteria in the gut aid in breakdown of feed, leading to better utilization of nutrients, which allows the animal to achieve higher nutrient digestibility (Cano et al., 2013). In swine, PRO primarily target the colon and cecum. This is because the largest number of bacteria in the gut are in these regions of the gastrointestinal tract (GIT). Including PRO in the diet causes competitive exclusion of

**Table 2.9.** Classification of commercial probiotics used in swine nutrition adapted from Liao and Nyachoti (2017)

| Categories | | Examples |
|---|---|---|
| Species / strain | Single-species / single-strain | Ecobiol (*Bacillus amyloliquefaciens*)<br>GutCare (*Bacillus subtilis*) |
| | Multi-species / multi-strain | PrimaLac (*Lactobacillus* spp., *E. faecium*, and *Bifidobacterium thermophilum*)<br>BIFILAC (*Bacillus mesentericus, B. coagulans, Enterococcus faecalis* and *Clostridium butyricum*) |
| Bacteria | Bacterial | *Lactobacillus spp.*<br>*Bacillus spp.* |
| | Non-bacterial | Actisaf Sc 47 (*Saccharomyces cerevisiae*)<br>*Saccharomyces boulardii* |
| Spores | Spore-forming | *Bacillus subtilis*<br>*Bacillus amyloliquefaciens* |
| | Non-spore-forming | *Bifidobacterium*<br>*Lactobacillus* |
| Origin | Allochthonous | Yeasts (i.e., *Saccharomyces cerevisiae*) |
| | Autochthonous | Bacteria already present in GIT (i.e., *Lactobacillus*) |

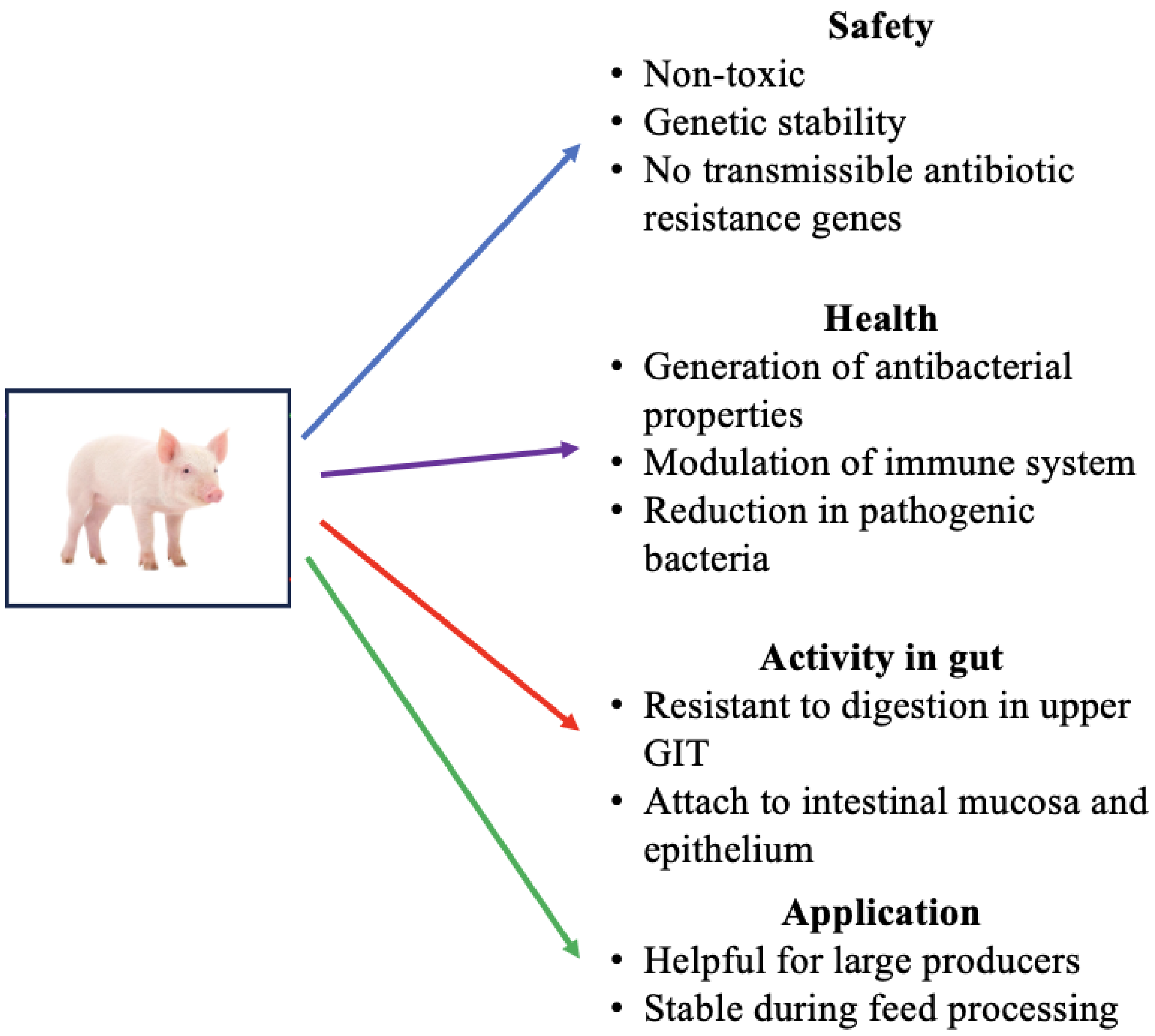
pathogens. This is accomplished by increasing the numbers of beneficial bacteria in the gut, while limiting the amount of non-beneficial, or pathogenic bacteria in the gut, balancing the gut microbiome (Cano et al., 2013). Beneficial bacteria in the gut lower pH, which creates an environment that pathogenic bacteria cannot survive in. This action can lead to reductions in illness prevalence in the pigs (Cano et al., 2013). Immune function can improve by supplementing PRO to swine diets by stimulating intestinal immune cells and cells attached to the wall of the GIT to modulate immune functions and homeostasis. They balance functions of dendric cells, macrophages, and T & B lymphocytes (Cano et al., 2023). All these cells aid in immune function; Dendric cells present pathogens to lymphocytes to regulate adaptive immune response (Liu, 2016), macrophages act as innate immune cells through phagocytosis and rid the gut of pathogenic substances (Lendeckel et al., 2022), and T & B cells are involved in antigen-specific immune responses, ridding the body of any unwanted bacteria (Cano et al., 2013).

There have been beneficial effects seen with PRO supplementation in both sows and weaned pigs. Dominigos et al. (2021) supplemented *Sacchomyces cerevisiae* to sows and reported increased production of colostrum and milk and increased SCFA production. In addition, Dominigos et al. (2021), Laskowska et al. (2019) and Ma et al. (2019) reported decreased numbers of stillborn piglets and increased piglet birthweights when *Sacchomyces cerevisiae* was supplemented into the diet. Jeong et al. (2015) supplemented a mixture of *Lactobacillus acidophilus* and *Bacillus licheniformis* to sows which resulted in increased voluntary feed intake and increased birthweights of piglets. Moreover, Hu et al. (2020) reported decreased E.coli attachment to the SI of sows when supplemented with a mixture of *Bacillus subtilis* and *Bacillus licheniformis*. Early weaned piglets are at higher risk of infection since the number of antibodies and nutrients from sow milk are reduced, however, there have been positive effects seen in early weaned piglets when PRO are supplemented into the diet. Yang et al. (2020) supplemented Lactobacillus plantarum which resulted in increased rate of weight gain due to better feed conversion. Tian et al. (2020) reported similar results when Lactobacillus reuteri was supplemented to early weaned pigs. In addition, Dávila-Ramírez et al. (2020) and Zhu et al. (2017) reported increased weight gain, increased beneficial bacteria in the gut, improved intestinal morphology and decreased colonization of pathogens in the gut of weaned pigs when supplemented with *Sacchomyces cerevisiae* at 0.3% yeast culture. At several stages of production, Jørgensen et al. (2016) supplemented *Bacillus subtilis* and *Bacillus licheniformis* into the diet of weaned piglets which resulted in increased weight gain, digestibility and feed efficiency compared to piglets without PRO supplementation. This shows that PRO supplementation can be beneficial to both sows and piglets in terms of performance and gut health.

Although there are many positive effects reported with PRO has been supplemented into the diets of pigs, there are some concerns about the safety of PRO supplementation in livestock. The Food and Drug Administration (FDA) approves certain PRO to be used in livestock nutrition (Cohen, 2018), however, there are concerns about bacterial PRO resistance among swine populations. Probiotics introduce new genes into the pig microbiome. Bacteria sold as PRO may have intrinsic or acquired resistance to antibiotics. Intrinsic resistance is defined as a strain’s typical resistance pattern (Cohen, 2018). This can cause increased health risks to pigs who develop infections from the PRO and may require further interferences (Cohen, 2018), which can cause detrimental effects due to increasing antimicrobial resistance among swine herds. Acquired resistance in PRO may show the presence of a gene capable of transferring antibiotic resistance to harmful bacteria (Egervärn et al., 2010), which can increase the rate at which infection occurs.

Siepert et al. (2014) reported that some strains of PRO can suppress immune response when pigs are exposed to disease. In this study, the researchers supplemented weaned pigs with *Enterococcus faeciem* which resulted in delayed Salmonella-induced proliferation of immune cells during active Salmonella infection. This shows that certain PRO strains can impair immune function and increase the risk of sepsis despite decreases in pathogenic bacteria in the gut (Siepert et al., 2014). Sepsis is a condition in which pathogenic bacteria enter the blood stream or other tissues which can lead to organ failure and death (Vintrych et al., 2022). Sepsis is more likely to occur in weaned pigs as inflammatory stress is increased, which can alter bacterial translocation due to increased permeability in the enterocyte (Cohen, 2018). Trevisi et al. (2008) reported increased translocation with *Bifidobacterium* and fructo-oligosaccharide treatment in weaned pigs which can increase the risk of sepsis. Therefore, there are several issues to consider when supplementing swine diets with PRO.

**Figure 2.9**. Expected outcomes of probiotic supplementation



(Adapted from Zhang et al., 2023)

## 2.10. MODE OF ACTION OF PROBIOTICS

There are several modes of action for PRO. Although they are not clearly defined, the speculated modes of action include gut microbiota modulation, immune system modulation, reduction in diarrhea, and modulation of nutrient digestibility (Latif et al., 2023; Liao and Nyachoti, 2017). The expected outcome of PRO supplementation is to prevent microbial imbalances in the gut and to improve GIT bacterial populations (Lescheid, 2014; Liao and Nyachoti, 2017). Supplementing diets with beneficial bacteria may improve immune function and resistance to diseases which can increase nutrient digestibility and absorption and increase performance potential. These effects from PRO supplementation can lead to reduced risk of disease, improved nutrient digestibility, and ultimately enhanced production performance.

When antibiotics were administered as a growth promoter in swine, consumer concern grew as the meat from the animals may contain antibiotics. In addition, as antibiotics are being banned from livestock production, it is possible that the meat may contain a higher number of pathogens leading to concerns for public health. Probiotics are being investigated as they have been shown to reduce the number of pathogenic bacteria and thereby minimize the risk of food-borne illnesses in humans due to contaminated meat. Probiotic bacteria are involved in competitive exclusion with pathogenic bacteria, where beneficial bacteria in the gut prevent attachment of pathogenic bacteria in the intestine, leading to decreased risk of disease. Beneficial bacteria compete with pathogenic bacteria in the gut for attachment sites and compete for energy and nutrients (Luykx et al., 2016; Liao and Nyachoti, 2017). The beneficial bacteria in the gut block adhesion sites for pathogenic bacteria, leading to an increased number of normal bacteria, and decreasing disease prevalence (Latif et al., 2023). Once PRO bacteria are established in the gut, they produce substances with antimicrobial properties that can prevent the proliferation of harmful bacteria (Hou et al., 2015; Liao and Nyachoti, 2017). Carbohydrates are metabolized into SCFA by PRO bacteria which drops the pH in the GIT, resulting in an environment where pathogenic bacteria cannot survive (Bajagai et al., 2016).

The immune system in pigs can be improved though PRO supplementation. The intestinal lumen contains a variety of substances which include beneficial bacteria, nutrients, toxins, and harmful bacteria (Latif et al., 2023). Innate immunity is a type of immunity that is present at birth and lasts throughout the entire life cycle and protects the body from harmful substances in the environment such as pathogens, toxins, and parasites (Roediger, B., and W. Weninger, 2015). A semi-permeable barrier is formed between the lumen and body tissues established by epithelial cells in the gut (Liao and Nyachoti, 2017). This barrier can lose functionality when experiencing illness or stress, which can cause substantial harm especially in weaned pigs (Lee et al., 2016; Liao and Nyachoti, 2017; Latif et al., 2023). Some PRO strains can improve the barrier function by modulating phosphorylation of tight junction and cytoskeletal proteins. This is thought to be accomplished by improving the expression of tight junction proteins by epithelial cells and altering the release of mucus and chlorides (Liao and Nyachoti, 2017). Probiotic bacteria have been shown to activate immune response and tolerance. Studies suggest that microbe-associated molecular patterns (MAMP) can interact with pattern recognition receptors (PRR) of GIT mucosa of the animal (Lebeer et al., 2010; Liao and Nyachoti, 2017). Intestinal epithelial cells and dendric cells can interact with gut microbes via PRRs that detect MAMPs such as bacterial DNA, thus differentiating between harmful and beneficial bacteria (Lebeer et al., 2010; Rachmileqitz et al., 2004; Liao and Nyachoti, 2017). Acquired immunity is either stimulated as a response to a foreign substance or bacteria, or when antibodies to particular bacteria are received from another source (Ballester et al., 2023).

Probiotics can establish anti-inflammatory cytokines and suppress pro-inflammatory cytokines from intestinal immune cells which can relieve gut upset (Cho et al., 2011). Specific strains of PRO such as *Lactobacillus* can act as immunomodulators by increasing macrophage activity, which can increase antibody levels and activate killer cells (Cho et al., 2011; Liao and Nyachoti, 2017). IgA and IgM antibodies can be increased by PRO bacteria by increasing the secretion of IL-6 from epithelial cells in the lumen, which aid in removing pathogens from the GIT (Czyżewska-Dors et al., 2018; Zhang et al., 2010). After PRO bacteria establish in the GIT, the acquired immune system can differentiate pathogenic bacteria from normal bacteria which increases the activity of lymphocytes to remove pathogens from the gut.

During the first 2 weeks after weaning, pigs are most susceptible to illness due to their immature immune system. Weaned pigs are no longer receiving antibodies from sow milk and their acquired immunity has not fully developed at this stage. Pathogenic bacteria produce enterotoxins that can cause excessive intestinal fluid loss which leads to increased incidence of diarrhea. Supplementing PRO can prevent attachment of pathogenic bacteria to the intestinal surface. Probiotics compete with pathogenic bacteria for adhesion sites on the intestinal mucosa and lower the pH of the gut which prevents colonization and proliferation of pathogenic bacteria that can cause diarrhea. In addition, some PRO strains produce antibacterial substances such as lactic and acetic acid that protect the gut from toxins produced by pathogenic bacteria (Ballester et al., 2023). Amines, which are produced by the decarboxylation of amino acids by coliform bacteria, can also be responsible for gut upset. Probiotic bacteria can help prevent gut irritation by supressing amine synthesis which can reduce the incidence of diarrhea. Minimal research has been done to investigate the effects of supplementing PRO to the sow diet on indices of piglet gut microbiome, however it is hypothesized that feeding PRO to sows may yield similar effects as feeding PRO to piglets directly.

When pigs have improper digestibility of nutrients, they are more susceptible to illness. Vitamins and minerals play a crucial role in maintaining the immune system, therefore when availability is reduced, the immune system may become compromised which can increase disease susceptibility. With decreased digestibility of nutrients, the animal will have reduced energy levels and therefore, may not have the energy to produce protein and new immune cells to fight infection (Straub, 2018). In addition, nutritional deficiencies can lead to health concerns such as brittle bones and muscle weakness, which may reduce feed intake and make pigs more susceptible to injuries and disease. Probiotics have been said to increase the digestibility of crude fibre, CP, and organic matter, which may be due to increased activity of digestive enzymes. Probiotics can increase digestibility by increasing fermentation and digestion of nutrients. When established in the gut, PRO increase the generation of digestive enzymes such as lipase amylase and protease (Suzer et al., 2008), thereby adding to existing enzymes produced by the host and improving digestion in the gut.

Two common PRO strains used in swine nutrition include *Bacillus subtilis* and *Bacillus amyloliquefaciens*. Both *B. subtilis* and *B. amyloliquefaciens* are from the genus Bacillus and are gram-positive, rod-shaped bacteria. Although these PRO strains are similar, they each have unique mechanisms of action. *Bacillus subtilis* is a common PRO strain used in swine nutrition because it has several modes of action which provide beneficial effects. *Bacillus subtilis* has the ability to form endospores which can survive the harsh conditions of the GIT such as acidic environments and bile salts, allowing the bacteria to reach the intestines where they can exert their beneficial effects (Nicholson et al., 2002). Once *B. subtilis* is in the gut, it can effectively colonize the intestinal tract by outcompeting pathogenic bacteria for substrates and binding sites in the intestinal tract (Ruiz Sella et al., 2021). In addition, this PRO enhances the production of specific antibodies and influences cytokine profiles which aid in modulating the immune system (Ruiz Sella et al., 2021). *Bacillus subtilis* can aid in digestive health by producing enzymes that assist in breakdown of carbohydrates and proteins, improving the nutrient absorption and reducing gastrointestinal inflammation (Ruiz Sella et al., 2021). *Bacillus amyloliquefaciens* is another PRO used in the swine industry. It produces high levels of amylase which can aid in the digestion of starches. This enzymatic activity helps break down carbohydrates more efficiently in the gut, contributing to better nutrient absorption, which can improve the health of the GIT (Rodrigues et al., 2024). Similar to *B. subtilis*, *B. amyloliquefaciens* produces antimicrobial peptides and compounds that can inhibit the growth and colonization of pathogenic bacteria, supporting a healthy balance of the gut microbiome (Rodrigues et al., 2024). This probiotic also forms biofilm which can help it adhere to the intestinal lining, promoting better colonization and stability in the gut. *Bacillus amyloliquefaciens* are often selected to be supplemented into swine diets due to their safety and efficacy, including their ability to survive GIT transit and interact positively with the gut microbiome of the host (Rodrigues et al., 2024). These strains of PRO were used in the current study for their beneficial effects on improving the gut health of weaned pigs and reducing PWD, which will be discussed in section 2.11 and 2.12.

## 2.11. EFFECT OF PROBIOTIC SUPPLEMENTATION IN SOW DIETS

Probiotics have traditionally been used in human nutrition in many different regions of the world for their health-promoting properties. There have been many beneficial effects seen in humans when PRO are included in the diet. Humans consume probiotics in many forms, such as fermented food and beverages and have been shown to have beneficial effects in human health. Probiotics can improve gut health by enhancing digestion of food by increasing the breakdown of nutrients. Some of these effects include prevention of acute diarrhea, Chron’s disease, and cardiovascular diseases (Bodke & Jogdand, 2022). In recent years, PRO have been investigated to test their effects in animal nutrition. Jeong et al. (2015), Tsukahara et al. (2018), and Saladrigas-Garćia et al. (2022) reported that piglets from sows with dietary supplemented Bacillus spp. PRO had increased birth and weaning weights compared to piglets from sows receiving a control diet. Wang et al. (2014), who used *Lactobacillus*, and Lipiński et al. (2012) who used *Saccharomyces cerevisiae* also reported increased birth and weaning weights compared to piglets from sows receiving control diets. Birth weights may be increased due to increased nutrient digestibility (Saladrigas-Garćia et al., 2022). Digestibility of nutrients can be improved by supplementing PRO into the diet because they balance the microbiota in the gut, leading to further breakdown of nutrients. These nutrients and energy can then be used for nourishing the unborn piglets, leading to higher birth weights (Liu et al., 2018). Higher weaning weights may be attributed to the quality and quantity of milk the piglets are receiving (Liu et al., 2018). Colostrum contains very important nutrients for the piglet and the quality of it can be either beneficial or detrimental to the piglet. Compared to milk, colostrum has a greater amount of protein and lower amounts of  fat and lactose (Gigli, 2016). The protein provides AA which are essential for intestinal growth and development. Piglets are born with weak GITs which hinders their ability to digest nutrients provided from the sow (Gigli, 2016). Receiving AA from colostrum is valuable to the piglet to prevent illnesses such as diarrhea. Piglets are born with weak immune systems, therefore ingesting AA from colostrum can provide a defense mechanism for when illness occurs, reducing the risk of severe illness and death. The protein in the colostrum also aids in growth performance, which is beneficial because piglets of larger size have a greater chance of survivability once weaned (Gigli, 2016). There is evidence that PRO supplementation can also improve milk quality and quantity. Some researchers have reported that sows receiving PRO supplementation produce a larger quantity of milk and colostrum which contained higher amounts of protein, lactose, and fat than those who did not receive PRO supplementation (Tsikahara et al., 2018; Saladrigas-Garćia et al., 2022; Chen et al. 2020). Thus, PRO can aid in the digestion of nutrients, therefore, more energy and nutrients can be used for milk production and composition (Theil & Hurley, 2015).

There have been studies to show improvements in litter sizes in sows receiving dietary PRO supplementation. Saladrigas-Garćis et al. (2022) saw an increase in total number of piglets born from sows receiving Bacillus spp. PRO supplementation compared to control. Although not significantly different, Wang et al. (2014), and Jeong et al. (2015) saw numerical increases in sows receiving dietary PRO supplementation in terms of total number of piglets born compared to control sows.

Pre-weaning mortality is one of the largest issues on farm. The average piglet mortality pre-weaning rate is approximately 10-20% (Tucker et al., 2021). Stillbirths are the most common cause of pre-weaning mortality (Jarratt et al., 2023). Sows are being selected and bred to produce a high number of piglets per litter (Jarrett et al., 2023). As the litter size increases, the number of stillbirths increase, which may be directly related to increased parturition time (Langendijk & Plush, 2019). Oxygen deprivation may occur if parturition is extended due to stretching and rupturing of the umbilical cord (Langendijk & Plush, 2019). Pre-weaning mortality can also be caused by congenital factors such as disease or weak piglets. Reducing pre-weaning mortality is vital in maintaining production and profit, and PRO supplementation has been as a strategy to reduce pre-weaning mortality. Jeong et al. (2015) and Tsukahara et al. (2018) reported that sows receiving Bacillus spp. PRO had a lower pre-weaning mortality rate than sows receiving control diets. It has been reported that sows receiving PRO supplementation produced a better quality colostrum, which can provide protein, fat, and immunoglobulins that can help the piglets fight infection (Chen et al., 2020). Since the piglets' immune system is not fully developed at this stage, the nutrients they are receiving from sow milk are vital in fighting infections and reducing the risk of death.

The wean to estrus period can have direct effects on the production and profit of a sow herd. The longer it takes for a sow to return to estrus after weaning, the less efficient she is, which can cost producers money. The average length of the wean-to-estrus period in sows is approximately three to seven days (Knox, 2021). There have been studies that show that PRO supplementation in sow diets can reduce the length of this period. Tsukahara et al. (2018) reported that sows receiving diets with Bacillus spp. PRO supplementation have shorter wean-to-estrus periods than sows without PRO supplementation. Sows receiving PRO may increase their feed consumption as PRO help to digest feed more efficiently. This can lead to less energy mobilization during lactation and can reduce the wean-to-estrus period (Tsukahara et al., 2018).

## 2.12. EFFECT OF PROBIOTIC SUPPLEMENTATION ON PIGLET GUT HEALTH AND WEANED PIG GUT HEALTH

The gut microbiome is a very complex environment that changes throughout the production cycle. Gut health can be improved by supplementing PRO to the diet of pigs at various stages of production. Probiotic supplementation aids in maintaining gut health by preventing the adhesion of pathogens to the intestinal mucosa. Within the complex ecosystem of the gut, probiotics compete with harmful pathogens for resources, and by colonizing the gut, probiotics create a barrier that impedes the attachment of pathogenic organisms to the intestinal lining (Pan et al., 2017). This is very important as harmful bacteria in the gut can attach to intestinal mucosa, colonize, and spread to other parts of the body via the circulatory system (Pan et al., 2017). As previously mentioned, ETEC is one of the most common pathogens that cause diarrheal diseases such as PWD, which can have detrimental effects for both the animal and the producer. Most of the research being conducted is on the effects of PRO on the gut health of weaned pigs due to the challenges these pigs face with PWD. Nursery pigs do not possess a mature immune system or GIT, making it difficult for them to overcome illness, and ETEC bacteria is the leading cause of PWD in weaned pigs, making research into prevention of ETEC colonization in the gut of most importance. Infection of PWD normally occurs within 2 weeks of weaning. Many types of PRO are being investigated for preventing adhesion and proliferation of pathogenic bacteria, such as *Bacillus spp., Lactobacillus spp., Saccharomyces spp.*, and *Enterococcus spp*. Pan et al. (2017) supplemented *Bacillus licheniformis* and *Saccharomyces cerevisiae* into diets of weaned pigs and found that pigs receiving diets with PRO supplementation had decreased cecal ETEC counts compared to control groups. Daudelin et al. (2011) supplemented *Pediococcus acidilactici* and *Saccharomyces cerevisiae boulardii* into the diet of weaned pigs and reported that pigs receiving diets supplemented with PRO had decreased ETEC attachment to ileal mucosa compared to pigs that did not receive PRO supplementation but had no influence on the number of ETEC bacteria in the gut. Lee et al. (2012) provided *Lactobacillus plantarum* PRO to weaned pigs under ETEC K88 challenge. They found that the number of pigs testing positive for ETEC infection was lower in pigs receiving a PRO-supplemented diet compared to pigs not receiving PRO supplementation. Another study with ETEC K88 challenge was conducted by Becker et al.

(2020) which reported that weaned pigs receiving a diet containing *Bacillus amyloliquefaciens* and *Bacillus subtilis* had increased ETEC shedding on day 3 post-inoculation, but reduced ETEC shedding on day 7 post-inoculation compared to pigs without PRO supplementation. Although there is an abundance of literature on the effects of PRO supplementation in diets of weaned pigs, there is little literature of the effects of PRO on suckling pig gut health. Hansen et al. (2022) inoculated suckling pigs with multi-species PRO containing *Bifidobacterium longum*, *Bifidobacterium breve*, *Lactobacillus rhamnosus*, and *Enterococcus faecium*. These researchers reported that piglets receiving PRO inoculation recovered quicker after ETEC challenge and had decreased ETEC shedding in feces compared to piglets given placebo inoculation. Another aspect worth investigating is maternal PRO supplementation on the effects of suckling pig gut health. Since there is evidence that PRO supplementation into weaned pig diets can reduce ETEC attachment and therefore reduce incidence of PWD, supplementation of PRO into the sow diet may also have beneficial effects in preventing adhesion of ETEC or other diarrhea inducing pathogens into the gut, preventing the presence of PWD in these piglets once weaned.

## 2.13. EFFECT OF CRUDE PROTEIN CONTENT AND PROBIOTIC SUPPLEMENTATION ON SOW AND PIGLET PERFORMANCE

As seen above, there are many beneficial effects seen for sow and piglet performance for either LCP or PRO supplementation in sow diets. There is limited research when it comes to the synergistic effects of LCP and PRO on sow and litter performance. More research needs to be done to test the effects of LCP diets supplemented with PRO to verify that the results seen from LCP diet and PRO supplementation are true when both are applied to the sow diet. This research could also be verified by conducting these trials on all stages of production to ensure the results seen in sows can also be seen in growing and finishing pigs. There is little research on the effects of LCP and PRO supplementation on gut health of suckling pigs. As mentioned above, there is a lot of evidence that reductions of CP and PRO supplementation can improve the health of the gut by modulating the balance of beneficial bacteria, leading to a healthier gut and increases in feed efficiency, leading to increased performance of these animals. If the effects seen by providing LCP diets and PRO supplementation separately to weaned pigs and sows are also seen when provided to sows on suckling pig gut health, this can prevent colonization of harmful bacteria in the gut, leading to decreases in incidence of PWD once the piglets are weaned, leading to healthier herds.

## 2.14. CONCLUSION

In conclusion, this review comprehensively explores the effects of LCP diets and probiotic supplementation in enhancing sow and suckling pig performance. Through an extensive examination of available literature, it is evident that both factors exert significant influences on the performance of pigs. It has been shown that LCP diets offer potential benefits in total number of piglets born, and increased birth and weaning weights. PRO supplementation is a promising strategy to improve reproductive performance of sows by improving milk composition, litter size, and growth performance of suckling pigs. However, the efficacy of these interventions is subject to multiple factors including probiotic strain, dosage, and environmental conditions. Hence, further research is warranted to elucidate the optimal integration of LCP diets and PRO supplementation, ensuring maximal benefits for sow and suckling pig performance. More research may be conducted to verify that these effects are seen when LCP diet and PRO supplementation are applied congruently, and that they are repeatable in different stages of production.

# CHAPTER 3: MANUSCRIPT

## 3.1. ABSTRACT

Post-weaning diarrhea poses a significant challenge to swine producers worldwide, impacting both economic viability and animal welfare. This condition primarily affects newly weaned animals due to a combination of factors including immature gut microbiota, stress from dietary changes, and pathogenic infections, which can lead to decreased feed efficiency, stunted growth, and increased mortality rates among affected animals which reduces overall productivity. Furthermore, the management of PWD often requires intensive veterinary intervention, including the use of antibiotics and supportive care, which adds to production costs and raises concerns regarding antimicrobial resistance. Strategies to mitigate PWD are being investigated to improve the productivity of swine herds. The objective of this study was to investigate the effects of CP content and supplementation of PRO in late-gestation and lactation sow diets on performance of sows and gut health of suckling pigs. A total of 120 TN70 pregnant sows (60 gilts and 60 2+ parity) were assigned to one of four dietary treatments in a 2 x 2 factorial design (2 CP levels, with or without PRO supplementation) from d 85 of gestation to d 21 of lactation. The diets consisted of 1) a corn-soybean meal (SBM)-based low CP (12% CP) diet; 2) LCP + 0.05% probiotic; 3) a corn-SBM-based high CP (15% CP) diet; and 4) HCP + 0.05% probiotic. Diets containing PRO contained *Bacillus subtilis* (2 x $10^9$ CFU/g) and *Bacillus amyloliquefaciens* (1 x $10^9$ CFU/g). Diets were fed in 2 phases (gestation (d 85-111 gestation) and lactation (d 1-21 lactation). Lactation diets contained 14% and 17% CP for LCP and HCP diets respectively. Sow BW and BF thickness were recorded on d 85 and 111 of gestation and on d 1 and 21 of lactation. Sow blood and milk were sampled on d 1 and 17 of lactation to analyze PUN concentration, and milk fat, protein, and lactose, respectively. Reproductive performance data was recorded on d 0

of lactation and sow feces were collected on d 12 of lactation for digestibility and microbial analysis. One piglet per sow close to the average BW for each litter was selected for blood collection to analyze PUN and fecal collection for microbial analysis. Data were analyzed using the PROC MIXED of SAS. There was an interaction ($P < 0.05$) between CP and PRO on the number of stillbirths, where the number of stillbirths did not differ between LCP and LCP-PRO groups, while the number decreased in sows fed HCP-PRO diet compared with the HCP diet. However, no difference was found in fat, protein, or lactose content in both colostrum (d 1) and milk (d 17). Piglets from the sows fed LCP diets showed heavier ($P < 0.05$) birth weights (1.58, 1.57, 1.53, and 1.52 kg for diets 1 to 4) than those in HCP groups, however, the average daily gain (ADG) of week 1 (188, 166, 187, and 187 g for diets 1 to 4) was less ($P < 0.05$) for piglets in LCP groups than HCP groups. There was an interaction ($P < 0.05$) between CP and PRO on piglet fecal DM, where HCP-PRO had higher fecal DM percentage than HCP, however, LCP and LCP-PRO groups did not differ. There was an interaction of CP and PRO on d 21 relative abundance of *E.coli*, where the abundance of was lower ($P < 0.05$) in piglets from sows fed HCP-PRO diets compared to piglets from sows fed HCP diets, however, piglets from sows fed LCP diet had a lower abundance ($P < 0.05$) of *E.coli* than piglets from sows receiving LCP-PRO diets. There was an interaction effect of CP and PRO on d 21 piglet *Lactobacillus* relative abundance, where piglets from sows receiving HCP diets had a lower ($P < 0.05$) relative abundance compared to piglets from sows receiving HCP-PRO diets, however, piglets from sows fed LCP-PRO diets had a lower ($P < 0.05$) relative abundance of *Lactobacillus* compared to piglets from sows fed LCP diets. On d 28, piglets from sows fed LCP diets had a decreased ($P < 0.05$) relative abundance of *E.coli* compared to pigs from sows fed HCP diets. In conclusion, feeding LCP diets supplemented with PRO to sows has the potential to improve reproductive performance of sows and improve gut health of suckling pigs by reducing *E.coli* and increasing *Lactobacillus* bacteria, therefore reducing the incidence of PWD.

## 3.2. INTRODUCTION

In swine production, optimizing dietary strategies to improve growth performance and health outcomes is paramount for sustainable and efficient operations. Since PWD is a major concern in swine populations, research is being done to investigate strategies to mitigate this disease. One such strategy under investigation is the use of LCP diets supplemented with PRO. This approach aims to balance nutritional requirements while enhancing gut health and overall performance in pigs, particularly during critical stages such as post-weaning.

Post-weaning represents a vulnerable period for pigs, characterized by a multitude of stressors including dietary changes, social group restructuring, and pathogen exposure. These stressors often lead to disruptions in GIT function and microbiota composition, contributing to post-weaning diarrhea and compromised growth rates. Traditional approaches to manage these challenges have typically relied on antibiotics, which pose concerns related to antimicrobial resistance and consumer preferences for antibiotic-free products. As an alternative, low-protein diets supplemented with probiotics have gained attention due to their potential to mitigate these challenges through multiple mechanisms. Probiotics have been shown to stabilize gut microbiota, improve nutrient utilization, and enhance immune responses in various species. By incorporating probiotics into low-protein diets, it is expected to not only reduce the environmental impact associated with high protein excretion but also to promote gut health and overall performance without compromising animal welfare.

Although positive effects have been seen from LCP diets and PRO separately, the synergistic effects of these treatments may improve both production efficiency and gut health greater than each treatment alone. Key parameters to evaluate include growth performance metrics such as feed efficiency and ADG, alongside health indicators such as gut microbiome and fecal dry matter. Since there is little research on the conjoint effects of LCP diet with PRO supplementation, the current study seeks to analyze the effects of LCP diets and PRO supplementation in the sow diet on sow performance and gut microbiome, and piglet performance and gut microbiome.

### 3.3. HYPOTHESES AND OBJECTIVES

The hypotheses of this study were:

1. The reproductive performance of sows receiving LCP diets will not differ from sows receiving HCP diets due to CAA supplementation in the LCP diet.
2. Growth performance of piglets from sows receiving LCP diets will not differ from piglets from sows receiving HCP diets.
3. Piglets from sows receiving diets with dietary PRO supplementation will have improved gut health than piglet from sows receiving diets without PRO supplementation.

The main objective of this study was to investigate the synergistic effects of LCP and PRO on performance of sows and gut health of suckling pigs.

The specific objectives of this study were:

1. To investigate the effects of CP content and dietary PRO supplementation on reproductive performance in late gestation and lactation sows.
2. To investigate the effects of CP and PRO supplementation in the sow diet on growth performance and gut health of suckling pigs.

## 3.4. MATERIALS AND METHODS

All methods and procedures used in this experiment were approved by the University of Manitoba Animal Care Committee (AC11406). Pigs were treated with care in accordance with guidelines proposed by the Canadian Council on Animal Care (2009).

### 3.4.1. ANIMALS, HOUSING, & EXPERIMENTAL DESIGN

A total of 120 sows [TN70 (60 gilts and 60 2+ parity); Topigs Norsvin, Winnipeg, MB, Canada] were selected from Glenlea Research Station at the University of Manitoba (Winnipeg, MB, Canada). Pigs were assigned to 1 of 4 treatments in s 2 x 2 factorial arrangement based on crude protein level and probiotic supplementation (with or without). The animal allotment was based on parity and backfat depth. Diets were formulated to be isoenergetic based on net energy (NE). Low crude protein diets were supplemented with DL-Methionine, L-Threonine, L-Tryptophan, and L-Isoleucine CAA. All other AA requirements were met in the diet therefore they were not supplemented.

Experimental diets were fed in mash form from d 85 of gestation until d 21 of lactation. All diets were formulated to meet the indispensable amino acid to lysine ratio based on AMINOPig® 1.0 (2011; Evonik Industries, Hanau-Wolfgang, Hessen, Germany). Diets were formulated in 2 levels (gestation (d 85-115) and lactation ration). Pigs were kept in group housing until d 111 of gestation. Sows were fed 3kg of feed once daily during the gestation period. On d 111 of gestation, pigs were moved and housed individually in farrowing crates until weaning. After farrowing, feed allowance was increased stepwise until ad libitum feed intake was achieved. Sows were induced to farrow by injecting prostaglandin on d 115 of gestation.

Farrowing rooms were mechanically ventilated, and the temperature was maintained at approximately 18 to 20°C.

One piglet [Tempo × TN70; Topigs Norsvin, Winnipeg, MB, Canada] of average litter body weight was selected for blood and fecal collections on d 21 of age, thus 30 replicates per treatment were collected. All piglets were cross-fostered within 24 to 48 hr after birth to achieve similar litter size and piglet body weight, and all piglets were breastfed from sows for 21 days.

### 3.4.2 EXPERIMENTAL DIETS

Four experimental diets were formulated based on 2 factors. The factors consisted of 2 CP levels (i.e., HCP and LCP) with or without probiotic supplementation. The CP concentrations were 12% and 15% for LCP and HCP for gestation, and 14% and 17% for LCP and HCP for lactation. The CP content of HCP diets was representative of the industry standard. Diets containing PRO supplementation included *B. subtillis* ($2 \times 10^9$ CFU/g) and *B. amyloliquefaciens* ($1 \times 10^9$ CFU/g) in powder form for a total inclusion rate of 0.05%. Spore counts were determined using NIR technology (Evonik Operations GmbH, Hanau-Wolfgang, Germany) and are presented in Table 4.1 and 4.2. Experimental diets were composed of soybean meal and corn. The amount of SBM was decreased in the LCP diets which were supplemented with CAA (L- Threonine, L-Tryptophan, and L- Isoleucine) to meet the indispensable AA to Lys recommendation by AMINOPig® 1.0 (2011; Evonik Industries, Hanau-Wolfgang, Hessen, Germany). All other nutrient requirements were met according to NRC (2012). Sows were fed gestation diets from d 85 of gestation until farrowing and were fed the lactation diets from farrowing until weaning. Sows had free access to water throughout the experimental period. The calculated and analyzed nutrient composition of gestation diets are shown in Table 4.1, and the calculated and analyzed nutrient composition of lactation diets are shown in Table 4.2.

### 3.4.3. SAMPLE COLLECTIONS AND MEASUREMENTS

Leftover feed was collected daily from sows during lactation to calculate average daily feed intake (**ADFI**). Sow BW and BF measurements were taken on d 85 and 111 of gestation, and d 0 and 21 of lactation to measure body condition score. The total number of piglets born, as well as piglets born alive, stillborn, and mummified were recorded on d 0 of lactation to measure reproductive performance. Blood was collected from each sow via jugular vein puncture on d 0 and 17 of lactation into a 10 mL heparinized vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, USA) to measure PUN concentration. Blood samples were centrifuged at 3,600 × g at 4˚C for 10 min to collect plasma from the blood sample. The plasma was kept at –80˚C until analysis was conducted. Sows were administered an intramuscular injection of 20 USP oxytocin (Rafter 8 products Inc., Calgary, AB, Canada) before milk collection. Colostrum and milk were collected from each sow manually on d 1 and 17 of lactation to measure fat, lactose, and protein content. Fat, protein, and lactose content were determined in the milk and colostrum samples by infrared spectroscopy using the MilkoScan7RM at the Horizon Lab Ltd. (Winnipeg, MB, Canada). Feces were collected from each sow via manual rectal stimulation one to two days post-farrowing to analyze fecal DM, microbial relative abundance, and digestibility of GE, CP and DM. Fecal samples were collected and stored in sterile bags and kept at -80˚C until analysis was performed.

Piglet BW was recorded on d 0, 7, 14, and 21 of lactation to assess growth performance. One piglet close to average litter BW was selected for blood and fecal collection for microbial analysis. Blood was collected via jugular vein puncture on d 21 of lactation into a 10 mL heparinized vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, USA) to measure PUN, and feces were collected via rectal swab. Four other pigs per litter were selected for fecal collection to measure fecal DM and digestibility of DM, CP, and GE. Fecal samples were collected and stored in sterile bags and kept at -80˚C until analysis was performed.

Microbial analysis of sow and piglet fecal samples was conducted following the method described by Koo et al. (2020). Total DNA extraction was conducted using a QIAamp 96 PowerFecal QIAcube HT kit (Qiagen, Venlo, Netherlands) following the protocol from the company. DNA quantity was measured using the Nanodrop 2000 spectrophotometer (ThermoScientific, Wilmington, DE, USA). Agarose gel electrophoresis was conducted to test the integrity of total DNA. qPCR was then preformed in duplicate reactions to ensure values were consistent. Bacterial primers used were based on the gene bank from the National Center for Biotechnology Information and are presented in Table 4.0.

**Table 4.0.** Primers used for qPCR analyses of fecal bacterial relative abundances

| Target | Forward strand | Reverse strand | Reference |
|---|---|---|---|
| Eubacteria | ACTCCTACGGGAGGCAG | GTATTACCGCGGCTGCTG | Yang et al. (2017) |
| Bifidobacterium | TCGCGTCYGGTGTGAAAG | CCACATCCAGCRTCCAC | Rinttila et al. (2004) |
| Lactobacillus | AGCAGTAGGGAATCTTCCA | CACCGCTACACATGGAG | Malinen et al. (2005) |
| E. coli subgroup 2 | GTTAATACCTTTGCTCATTGA | ACCAGGGTATCTAATCCTGTT | Malinen et al. (2003) |
| Clostridium perfringens | CGCATAACGTTGAAAGATGG | CCTTGGTAGGCCGTTACCC | Wise and Siragusa (2005) |

### 3.4.4. CHEMICAL ANALYSES

Diet samples were collected and analyzed after mixing to ensure they met the indispensable AA to Lys recommendation of AMINOPig® 1.0 (2011; Evonik Industries, Hanau-Wolfgang, Hessen, Germany), and to ensure consistency between periods. All diet samples were finely ground and analyzed for dry matter (DM), CP and AA. Dry matter was measured following AOAC (method 934.01; 2006), and CP was calculated by determining nitrogen with the combustion method and using the LECO N analyzer (model CNS-2000; LECO Corp., St. Joseph, MI) (nitrogen x 6.25). Amino acids in diet samples were determined by ion-exchange chromatography with post-column derivatization with ninhydrin. Amino acids were hydrolyzed for 24 hours at 110 °C in 6 N HCl. Samples with sulfur-containing AA were oxidized before hydrolyzation using performic acid (Commission Directive, 1998). The quantity of AA was determined by the standard internal method in which absorption of reaction products with ninhydrin at 570 nm is measured. Tryptophan was determined by HPLC with fluorescence detection after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110˚C (Commission Directive, 2000).

**Table 4.1.** Composition of experimental diets for late gestation sows[1]

| | Gestation diets | | | |
|---|---|---|---|---|
| | HCP | HCP-Pro | LCP | LCP-Pro |
| Ingredients, % | | | | |
| Corn | 42.06 | 42.06 | 51.18 | 51.18 |

| | | | | |
|---|---|---|---|---|
| Barley | 20.00 | 20.00 | 20.00 | 20.00 |
| Wheat bran | 10.00 | 10.00 | 10.00 | 10.00 |
| Sugar beet pulp | 10.00 | 10.00 | 10.00 | 10.00 |
| Soybean meal | 13.68 | 13.68 | 4.72 | 4.72 |
| Canola oil | 0.81 | 0.81 | 0.07 | 0.07 |
| Limestone | 1.27 | 1.27 | 1.28 | 1.28 |
| Monocalcium phosphate | 1.25 | 1.25 | 1.36 | 1.36 |
| Iodized salt | 0.40 | 0.40 | 0.40 | 0.40 |
| Vit-Min premix | 0.50 | 0.50 | 0.50 | 0.50 |
| Lys-HCl | 0.02 | 0.02 | 0.29 | 0.29 |
| DL-Methionine | 0.01 | 0.01 | 0.06 | 0.06 |
| L-Threonine | - | - | 0.11 | 0.11 |
| L-Tryptophan | - | - | 0.03 | 0.03 |
| L-Isoleucine | - | - | 0.01 | 0.01 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated nutrient composition, % | | | | |
| Net energy, kcal/kg | 2,370 | 2,370 | 2,370 | 2,370 |
| Crude protein | 15.0 | 15.0 | 12.0 | 12.0 |
| SID[3] amino acids | | | | |
| SID Arginine | 0.81 | 0.81 | 0.55 | 0.55 |
| SID Histidine | 0.34 | 0.34 | 0.26 | 0.26 |
| SID Lysine | 0.59 | 0.59 | 0.59 | 0.59 |
| SID Methionine | 0.21 | 0.21 | 0.22 | 0.22 |
| SID Methionine + Cysteine | 0.42 | 0.42 | 0.39 | 0.39 |
| SID Threonine | 0.42 | 0.42 | 0.41 | 0.41 |
| SID Tryptophan | 0.15 | 0.15 | 0.13 | 0.13 |
| SID Isoleucine | 0.49 | 0.49 | 0.35 | 0.35 |
| SID Leucine | 1.06 | 1.06 | 0.86 | 0.86 |
| SID Valine | 0.58 | 0.58 | 0.43 | 0.43 |
| SID Phenylalanine | 0.60 | 0.60 | 0.44 | 0.44 |
| Total amino acids | | | | |
| Arginine | 0.90 | 0.90 | 0.63 | 0.63 |
| Histidine | 0.40 | 0.40 | 0.32 | 0.32 |
| Lysine | 0.72 | 0.72 | 0.70 | 0.70 |
| Methionine | 0.25 | 0.25 | 0.25 | 0.25 |
| Methionine + Cysteine | 0.50 | 0.50 | 0.47 | 0.47 |
| Threonine | 0.55 | 0.55 | 0.53 | 0.53 |
| Tryptophan | 0.18 | 0.18 | 0.16 | 0.16 |
| Isoleucine | 0.58 | 0.58 | 0.43 | 0.43 |
| Leucine | 1.25 | 1.25 | 1.03 | 1.03 |
| Valine | 0.72 | 0.72 | 0.56 | 0.56 |
| Phenylalanine | 0.71 | 0.71 | 0.54 | 0.54 |

|  |  |  |  |  |
|---|---|---|---|---|
| Analyzed nutrient composition, % |  |  |  |  |
| Crude protein | 15.0 | 14.8 | 12.2 | 12.3 |
| Arginine | 0.89 | 0.85 | 0.63 | 0.63 |
| Histidine | 0.39 | 0.37 | 0.30 | 0.30 |
| Lysine | 0.73 | 0.69 | 0.71 | 0.71 |
| Methionine | 0.26 | 0.25 | 0.25 | 0.26 |
| Methionine + Cysteine | 0.53 | 0.51 | 0.47 | 0.47 |
| Threonine | 0.57 | 0.53 | 0.51 | 0.52 |
| Tryptophan | 0.19 | 0.19 | 0.16 | 0.17 |
| Isoleucine | 0.59 | 0.56 | 0.43 | 0.43 |
| Leucine | 1.30 | 1.23 | 1.06 | 1.06 |
| Valine | 0.73 | 0.69 | 0.56 | 0.56 |
| Phenylalanine | 0.73 | 0.70 | 0.56 | 0.55 |
| Spore count |  |  |  |  |
| *Bacillus Subtilis* | <100 | 1.4E+6 | <100 | 1.6E+6 |
| *Bacillus Amyloliquefaciens* | <100 | 6E+5 | <100 | 8E+5 |

[1] HCP = high crude protein diet without probiotics supplementation; HCP-Pro = high crude protein diet with probiotics supplementation; LCP = low crude protein diet without probiotics supplementation; LCP-Pro = low crude protein diet with probiotics supplementation.

[2] Provided the following nutrients (per kg of air-dry diet): Vitamins: A, 4,000 IU, $D_3$ 800 IU, E, 44 mg, K, 0.5 mg, $B_1$, 1 mg, $B_2$, 3.75 mg, $B_6$, 1 mg, $B_{12}$, 15 µg, calcium pantothenate, 12 mg, folic acid, 1.3 mg, niacin, 10 mg, biotin, 0.2 mg. Minerals: Cu, 10 mg (as copper sulphate), iodine, 0.14 mg (as calcium iodate), iron, 80 mg (as ferrous sulphate), Mn, 25 mg (as manganese oxide), Se, 0.15 mg (as sodium selenite), Zn, 100 mg (as zinc oxide).

[3] SID = standardized ileal digestible.

**Table 4.2.** Composition of experimental diets for lactation sows[1]

|  | Lactation diets |  |  |  |
|---|---|---|---|---|
|  | HCP | HCP-Pro | LCP | LCP-Pro |
| Ingredients, % |  |  |  |  |

|  |  |  |  |  |
|---|---|---|---|---|
| Corn | 55.11 | 55.11 | 64.59 | 64.59 |
| Barley | 10.00 | 10.00 | 10.00 | 10.00 |
| Wheat bran | 5.00 | 5.00 | 5.00 | 5.00 |
| Sugar beet pulp | 5.00 | 5.00 | 5.00 | 5.00 |
| Soybean meal | 19.96 | 19.96 | 10.45 | 10.45 |
| Canola oil | 1.34 | 1.34 | 0.46 | 0.46 |
| Limestone | 1.05 | 1.05 | 1.06 | 1.06 |
| Monocalcium phosphate | 1.24 | 1.24 | 1.35 | 1.35 |
| Iodized salt | 0.40 | 0.40 | 0.40 | 0.40 |
| Vit-Min premix | 0.50 | 0.50 | 0.50 | 0.50 |
| Lys-HCl | 0.19 | 0.19 | 0.48 | 0.48 |
| DL-Methionine | 0.05 | 0.05 | 0.13 | 0.13 |
| L-Threonine | 0.08 | 0.08 | 0.21 | 0.21 |
| L-Tryptophan | 0.03 | 0.03 | 0.08 | 0.08 |
| L-Valine | 0.05 | 0.05 | 0.22 | 0.22 |
| L-Isoleucine | - | - | 0.07 | 0.07 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated nutrient composition, % |  |  |  |  |
| Net energy, kcal/kg | 2,550 | 2,550 | 2,550 | 2,550 |
| Crude protein | 17.1 | 17.1 | 14.1 | 14.1 |
| SID[3] amino acids |  |  |  |  |
| SID Arginine | 0.95 | 0.95 | 0.68 | 0.68 |
| SID Histidine | 0.38 | 0.38 | 0.30 | 0.30 |
| SID Lysine | 0.85 | 0.85 | 0.85 | 0.85 |
| SID Methionine | 0.28 | 0.28 | 0.32 | 0.32 |
| SID Methionine + Cysteine | 0.51 | 0.51 | 0.51 | 0.51 |
| SID Threonine | 0.58 | 0.58 | 0.58 | 0.58 |
| SID Tryptophan | 0.19 | 0.19 | 0.19 | 0.19 |
| SID Isoleucine | 0.58 | 0.58 | 0.50 | 0.50 |
| SID Leucine | 1.27 | 1.27 | 1.06 | 1.06 |
| SID Valine | 0.72 | 0.72 | 0.72 | 0.72 |
| SID Phenylalanine | 0.71 | 0.71 | 0.54 | 0.54 |
| Total amino acids |  |  |  |  |
| Arginine | 1.03 | 1.03 | 0.75 | 0.75 |
| Histidine | 0.44 | 0.44 | 0.35 | 0.35 |
| Lysine | 0.98 | 0.98 | 0.96 | 0.96 |
| Methionine | 0.31 | 0.31 | 0.35 | 0.35 |
| Methionine + Cysteine | 0.60 | 0.60 | 0.59 | 0.59 |
| Threonine | 0.70 | 0.70 | 0.68 | 0.68 |
| Tryptophan | 0.22 | 0.22 | 0.22 | 0.22 |
| Isoleucine | 0.68 | 0.68 | 0.58 | 0.58 |
| Leucine | 1.46 | 1.46 | 1.23 | 1.23 |
| Valine | 0.85 | 0.85 | 0.83 | 0.83 |

|  |  |  |  |  |
|---|---|---|---|---|
| Phenylalanine | 0.82 | 0.82 | 0.63 | 0.63 |
| Analyzed nutrient composition, % |  |  |  |  |
| Crude protein | 17.08 | 17.06 | 14.43 | 14.08 |
| Arginine | 1.02 | 1.01 | 0.74 | 0.76 |
| Histidine | 0.43 | 0.43 | 0.34 | 0.34 |
| Lysine | 0.99 | 0.97 | 0.90 | 0.97 |
| Methionine | 0.31 | 0.31 | 0.33 | 0.34 |
| Methionine + Cysteine | 0.60 | 0.60 | 0.56 | 0.58 |
| Threonine | 0.69 | 0.70 | 0.71 | 0.69 |
| Tryptophan | 0.23 | 0.23 | 0.22 | 0.22 |
| Isoleucine | 0.68 | 0.69 | 0.57 | 0.58 |
| Leucine | 1.50 | 1.49 | 1.28 | 1.27 |
| Valine | 0.84 | 0.85 | 0.81 | 0.83 |
| Phenylalanine | 0.82 | 0.82 | 0.65 | 0.66 |
| Spore count |  |  |  |  |
| *Bacillus Subtilis* | <100 | 1.2E+6 | 1000 | 1.6E+6 |
| *Bacillus Amyloliquefaciens* | <100 | 8.6E+5 | <100 | 8.0E+5 |
| Analyzed nutrient composition (Ti diet), % |  |  |  |  |
| Crude protein | 17.05 | 17.18 | 14.38 | 14.13 |
| Arginine | 1.04 | 1.02 | 0.76 | 0.74 |
| Histidine | 0.44 | 0.43 | 0.35 | 0.34 |
| Lysine | 0.98 | 0.98 | 0.93 | 0.99 |
| Methionine | 0.32 | 0.31 | 0.34 | 0.36 |
| Methionine + Cysteine | 0.60 | 0.60 | 0.58 | 0.59 |
| Threonine | 0.69 | 0.70 | 0.63 | 0.67 |
| Tryptophan | 0.22 | 0.23 | 0.22 | 0.23 |
| Isoleucine | 0.71 | 0.70 | 0.59 | 0.60 |
| Leucine | 1.53 | 1.51 | 1.29 | 1.24 |
| Valine | 0.86 | 0.86 | 0.84 | 0.87 |
| Phenylalanine | 0.85 | 0.83 | 0.65 | 0.64 |
| Spore count (Ti diet) |  |  |  |  |
| *Bacillus Subtilis* | 200 | <100 | <100 | <100 |
| *Bacillus Amyloliquefaciens* | <100 | 3.7E+6 | <100 | 3.5E+6 |

[1] HCP = high crude protein diet without probiotics supplementation; HCP-Pro = high crude protein diet with probiotics supplementation; LCP = low crude protein diet without probiotics supplementation; LCP-Pro = low crude protein diet with probiotics supplementation.

[2] Provided the following nutrients (per kg of air-dry diet): Vitamins: A, 4,000 IU, $D_3$ 800 IU, E, 44 mg, K, 0.5 mg, $B_1$, 1 mg, $B_2$, 3.75 mg, $B_6$, 1 mg, $B_{12}$, 15 µg, calcium pantothenate, 12 mg, folic acid, 1.3 mg, niacin, 10 mg, biotin, 0.2 mg. Minerals: Cu, 10 mg (as copper sulphate), iodine, 0.14 mg (as calcium iodate), iron, 80 mg (as ferrous sulphate), Mn, 25 mg (as manganese oxide), Se, 0.15 mg (as sodium selenite), Zn, 100 mg (as zinc oxide).

[3]SID = standardized ileal digestible.

### 3.4.5. STATISTICAL ANALYSIS

Data were analyzed using the MIXED procedure of SAS (version 9.4; SAS Institute Inc., Cary, NC). The main effects of the model were CP content, PRO supplementation, and their interaction, and period and replication were considered random effects. For sow data, the experimental unit was a sow. For piglet growth performance data, pen was considered the experimental unit, and for piglet blood collection, the individual piglet selected was considered the experimental unit. Significant differences were considered at $P \leq 0.05$, and tendencies were observed at $0.05 < P \leq 0.10$.

## 3.5. RESULTS

The analyzed nutrient composition of both gestation and lactation diets was within range of calculated values. Gestation diets contained 15.0%, 14.8%, 12.2%, and 12.3% CP, and lactation diets contained 17.08%, 17.06%, 14.43%, and 14.08% CP for HCP, HCP-PRO, LCP, and LCP-PRO respectively. The calculated values for diets 1 – 4 were 15% CP for HCP diets and 12% CP for LCP diets in gestation, and 17.1% CP for HCP diets and 14.1% CP for LCP diets in lactation.

The digestibility of GE, CP and DM did not differ ($P > 0.05$) due to CP content or PRO supplementation.

### 3.5.1. SOW BODY CONDITION & REPRODUCTIVE PERFORMANCE

The results for sow body weight, backfat thickness, and ADFI are presented in Table 5.1. There were no effects of CP content or PRO supplementation on sow BW and BF on d 85 and 111 of gestation ($P > 0.10$), or d 1 and 21 of lactation ($P > 0.10$). Lactation feed disappearance did not differ among treatments ($P > 0.05$).

**Table 5.1**. Effects of CP content with or without probiotic supplementation on sow body weight, backfat thickness, and average daily feed intake

| | | High CP | | Low CP | | | *P*-values[1] | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | Pro: | - | + | - | + | SEM | CP | PRO | CP x PRO |
| Day 85 of gestation | | | | | | | | | |
| Body weight, kg | | 247 | 251 | 250 | 248 | 7.64 | 0.930 | 0.771 | 0.556 |
| Backfat thickness, mm | | 15.2 | 15.0 | 15.3 | 14.8 | 0.491 | 0.878 | 0.406 | 0.656 |
| Day 111 of gestation | | | | | | | | | |
| Body weight, kg | | 274 | 278 | 276 | 273 | 6.33 | 0.672 | 0.929 | 0.426 |
| Backfat thickness, mm | | 16.0 | 15.0 | 15.5 | 15.3 | 0.388 | 0.813 | 0.075 | 0.206 |
| Day 1 of lactation | | | | | | | | | |
| Body weight, kg | | 253 | 254 | 254 | 247 | 5.94 | 0.461 | 0.542 | 0.427 |
| Backfat thickness, mm | | 15.6 | 14.7 | 15.2 | 15.3 | 0.510 | 0.882 | 0.224 | 0.184 |
| Day 21 of lactation | | | | | | | | | |
| Body weight, kg | | 244 | 242 | 239 | 237 | 5.99 | 0.297 | 0.738 | 0.965 |
| Backfat thickness, mm | | 14.2 | 13.2 | 14.0 | 13.7 | 0.362 | 0.716 | 0.078 | 0.400 |
| Average daily feed intake, kg | | 5.58 | 5.44 | 5.56 | 5.57 | 4.43 | 0.453 | 0.468 | 0.831 |

[1] CP, main effect of crude protein levels; Pro, main effect of probiotics supplementation; CP × Pro, interactive effect of crude protein levels and probiotics; BW = body weight; ADG = average daily gain.

The results for reproductive performance are listed in table 5.2. There was no effect of CP content or PRO supplementation on the number of total born or live born piglets ($P > 0.10$), however, there was an interaction effect on the number of stillborn piglets, where the number of stillborn piglets did not differ ($P > 0.10$) between the LCP and the LCP-PRO group, while the number of stillborn piglets was lower ($P < 0.05$) in HCP-PRO group compared to the HCP group. There was also an interaction effect of CP content and PRO supplementation in the number of piglets born mummified, where the number of mummified piglets was lower ($P < 0.05$) in LCP-PRO compared to LCP, but did not differ ($P > 0.10$) between HCP and HCP-PRO.

**Table 5.2.** Effects of CP level with or without PRO supplementation on reproductive performance of sows

|  | High CP |  | Low CP |  |  | *P*–values[1] |  |  |
|---|---|---|---|---|---|---|---|---|
| Item Pro: | - | + | - | + | SEM | CP | PRO | CP x PRO |
| Total born | 16.6 | 16.4 | 16.6 | 16.8 | 0.528 | 0.669 | 0.996 | 0.780 |
| Live born | 14.9 | 15.1 | 15.2 | 15.7 | 0.465 | 0.341 | 0.494 | 0.768 |
| Stillborn | 1.41 | 0.44 | 0.56 | 0.80 | 0.205 | 0.214 | 0.066 | **0.003** |
| Mummified | 0.15 | 0.33 | 0.38 | 0.11 | 0.097 | 0.987 | 0.648 | **0.023** |

[1] CP, main effect of crude protein levels; PRO, main effect of probiotics supplementation; CP × Pro, interactive effect of crude protein levels and probiotics

### 3.5.2. SOW MILK COMPOSITION & PLASMA UREA NITROGEN

Colostrum (lactation d 1) and milk (lactation d 17) composition are reported in Table 5.3. Fat, protein, and lactose content did not differ ($P > 0.05$) based on dietary CP content and PRO supplementation. Sows receiving LCP diets had lower ($P < 0.05$) PUN concentrations on both day 1 and 17 of lactation compared to sows receiving HCP diets.

**Table 5.3.** Effects of CP content with or without PRO supplementation on colostrum and milk composition and PUN concentrations

| | High CP | | Low CP | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| Item Pro: | - | + | - | + | SEM | CP | PRO | CP x PRO |
| Colostrum (d 1 lactation), % | | | | | | | | |
| Fat | 6.92 | 7.56 | 6.81 | 6.62 | 0.404 | 0.200 | 0.583 | 0.310 |
| Protein | 7.88 | 7.49 | 7.71 | 8.05 | 0.550 | 0.689 | 0.958 | 0.456 |
| Lactose | 5.20 | 5.06 | 5.25 | 5.10 | 0.121 | 0.691 | 0.215 | 0.964 |
| Milk (d 17 lactation), % | | | | | | | | |
| Fat | 7.19 | 7.35 | 7.29 | 7.40 | 0.280 | 0.757 | 0.588 | 0.904 |
| Protein | 4.64 | 4.57 | 4.52 | 4.62 | 0.087 | 0.692 | 0.844 | 0.305 |
| Lactose | 7.11 | 8.19 | 7.18 | 7.14 | 0.082 | 0.811 | 0.531 | 0.152 |
| Plasma urea nitrogen mmol/L | | | | | | | | |
| Day 1 lactation | 4.25 | 4.22 | 3.09 | 3.04 | 1.53 | **0.001** | 0.797 | 0.953 |
| Day 20 lactation | 5.18 | 4.99 | 2.58 | 2.87 | 0.168 | **0.001** | 0.760 | 0.161 |

[1] CP, main effect of crude protein levels; Pro, main effect of probiotics supplementation; CP × Pro, interactive effect of crude protein levels and probiotics

### 3.5.3. SOW FECAL DRY MATTER AND BACTERIAL RELATIVE ABUNDANCES

The effects of CP content and PRO supplementation on sow fecal DM and microbiome relative abundance are presented in Table 5.4. Sow fecal dry matter did not statistically differ ($P > 0.10$) between treatments, however there was a tendency for sows receiving LCP diets to have a higher ($P = 0.058$) fecal DM percentage compared to sows receiving HCP diets. There were no statistical differences ($P > 0.10$) in relative abundances of *Lactobacillus* or *Bifidobacterium*, however, there was a tendency ($P = 0.069$) for a lower relative abundance of *Lactobacillus* in sows fed diets containing PRO compared to sows fed diets without PRO supplementation.

**Table 5.4.** Effects of different crude protein (CP) level with or without probiotics (PRO) supplementation on bacterial relative abundance in sows and piglets

| | | High CP | | Low CP | | | P-values | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | Pro: | - | + | - | + | SEM | CP | PRO | CP x PRO |
| Fecal DM, % | | | | | | | | | |
| d 1 lactation | | 29.9 | 30.1 | 30.7 | 31.1 | 0.248 | 0.058 | 0.543 | 0.833 |
| Relative abundance, fold change | | | | | | | | | |
| *Lactobacillus* | | 0.26 | 0.41 | 0.38 | 0.39 | 0.046 | 0.224 | **0.069** | 0.139 |
| *Bifidobacterium* | | 0.03 | 0.02 | 0.02 | 0.02 | 0.006 | 0.370 | 0.161 | 0.509 |

### 3.5.4. PIGLET GROWTH PERFORMANCE

The effects of CP content and PRO supplementation in the sow diet on piglet BW and ADG are shown in Table 5.4. There were no effects ($P > 0.10$) of CP content or PRO supplementation in the sow diet on d 1or 14 piglet BW, however, there was an effect on piglet birth weights, where piglets from sows fed LCP diets had heavier ($P < 0.05$) birth weights than piglets from sows receiving HCP diets. Piglet BW on d 21 tended to be higher ($P = 0.078$) in piglets from sows receiving HCP diets compared to piglets from sows receiving LCP diets. Moreover, there was no effect ($P > 0.10$) on week 2 or 3 of piglet ADG due to CP content or PRO supplementation in the sow diet, however, piglets from sows receiving LCP diets had lower ($P < 0.05$) ADG in week 1 compared to piglets from sows receiving HCP diets.

**Table 5.5.** Effects of CP content with or without PRO supplementation in the sow diet on growth performance of suckling pigs

| | | High CP | | Low CP | | | P-values | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | Pro: | - | + | - | + | SEM | CP | PRO | CP x PRO |
| BW, kg | | | | | | | | | |
| Day 1 | | 1.53 | 1.52 | 1.58 | 1.57 | 0.021 | **0.028** | 0.598 | 0.996 |
| Day 7 | | 2.69 | 2.71 | 2.68 | 2.51 | 0.091 | 0.168 | 0.326 | 0.189 |
| Day 14 | | 4.56 | 4.70 | 4.56 | 4.38 | 0.105 | 0.133 | 0.850 | 0.128 |
| Day 21 | | 6.58 | 6.70 | 6.56 | 6.21 | 0.147 | **0.078** | 0.440 | 0.117 |
| ADG, g/day | | | | | | | | | |
| Week 1 | | 187 | 187 | 181 | 166 | 7.463 | **0.021** | 0.231 | 0.204 |
| Week 2 | | 268 | 282 | 267 | 269 | 6.026 | 0.229 | 0.190 | 0.293 |
| Week 3 | | 281 | 283 | 269 | 271 | 9.185 | 0.118 | 0.769 | 0.987 |

[1] CP, main effect of crude protein levels; Pro, main effect of probiotics supplementation; CP × Pro, interactive effect of crude protein levels and probiotics; BW = body weight; ADG = average daily gain.

### 3.5.5. PIGLET PLASMA UREA NITROGEN, FECAL DRY MATTER & MICROBIAL ANALYSIS

Results for piglet PUN concentrations and fecal DM are presented in Table 5.6. There was no effect ($P > 0.10$) of CP content or PRO supplementation in the sow diet on piglet PUN concentrations on d 21. There was an interaction effect of CP content and PRO supplementation in the sow diet on d 21 of piglet fecal DM, where piglets from sows receiving HCP-PRO diet had a higher ($P < 0.05$) percentage of fecal DM compared to piglets from sows receiving HCP diet, however, there were no differences ($P > 0.10$) seen between LCP and LCP-PRO groups. On d 28, the fecal DM percentage was greater ($P < 0.05$) in piglets from sows receiving diets with PRO supplementation compared to piglets from sows without PRO supplementation.

Results of piglet microbiome relative abundance is shown in Table 5.7. There was an interaction effect of CP and PRO on d 21, where the abundance of *E.coli* was lower ($P < 0.05$) in piglets from sows fed HCP-PRO diets compared to piglets from sows fed HCP diets, however, piglets from sows fed LCP diet had a lower abundance ($P < 0.05$) of *E.coli* than piglets from sows receiving LCP-PRO diets. There was also an interaction effect of CP and PRO on d 21, where piglets from sows receiving HCP diets had a lower ($P < 0.05$) relative abundance of *Lactobacillus* compared to piglets from sows receiving HCP-PRO diets, however, piglets from sows fed LCP-PRO diets had a lower ($P < 0.05$) relative abundance of *Lactobacillus* compared to piglets from sows fed LCP diets. On d 28, pigs from sows fed LCP diets had a decreased ($P < 0.05$) relative abundance of *E.coli* compared to pigs from sows fed HCP diets. There was an interaction effect of CP and PRO on d 28 where pigs from sows receiving HCP diets had a decreased ($P < 0.05$) relative abundance of *Clostridium perfringens* compared to sows receiving

HCP-PRO diet. No differences were seen between LCP and LCP-PRO groups on d 28 for *Clostridium perfringens* relative abundance.

**Table 5.6**. Effect of crude protein content and probiotic supplementation in the sow diet on plasma urea nitrogen concentrations and fecal DM of suckling pigs

| | | High CP | | Low CP | | | P-values | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | Pro: | - | + | - | + | SEM | CP | PRO | CP x PRO |
| Plasma urea nitrogen, mmol/L | | | | | | | | | |
| d 21 | | 1.92 | 1.68 | 1.65 | 1.58 | 0.127 | 0.118 | 0.204 | 0.454 |
| Fecal DM, % | | | | | | | | | |
| d 21 | | 42.1 | 46.2 | 44.1 | 41.7 | 0.958 | 0.256 | 0.424 | **0.002** |
| d 28 | | 27.7 | 28.1 | 25.6 | 28.7 | 0.392 | 0.340 | **0.029** | 0.080 |

**Table 5.7.** Effect of crude protein (CP) content and probiotic (PRO) supplementation in the sow diet on piglet bacterial relative abundances

| | High CP | | Low CP | | | P-values[1] | | |
|---|---|---|---|---|---|---|---|---|
| Item Pro: | - | + | - | + | SEM | CP | PRO | CP x PRO |
| d 21 | | | | | | | | |
| *E. coli* | $1.13^{a}$ | $0.54^{ab}$ | $0.44^{b}$ | $0.97^{ab}$ | 0.231 | 0.523 | 0.880 | **0.009** |
| *Clostridium perfringens* | 1.65 | 1.15 | 1.41 | 2.44 | 0.645 | 0.422 | 0.685 | 0.245 |
| *Lactobacillus* | $3.78^{b}$ | $8.88^{ab}$ | $9.65^{a}$ | $5.11^{ab}$ | 1.911 | 0.584 | 0.883 | **0.015** |
| d 28 | | | | | | | | |
| *E. coli* | 6.47 | 14.32 | 0.89 | 0.55 | 3.287 | **0.005** | 0.259 | 0.219 |
| *Clostridium perfringens* | $0.86^{a}$ | $0.33^{b}$ | $0.28^{b}$ | $0.40^{b}$ | 0.167 | 0.031 | 0.075 | **0.007** |
| *Lactobacillus* | 1.81 | 1.62 | 1.38 | 1.48 | 0.411 | 0.485 | 0.910 | 0.731 |

[1] CP, main effect of crude protein levels; Pro, main effect of probiotics supplementation; CP × Pro, interactive effect of crude protein levels and probiotics.

## 3.6. DISCUSSION

Antibiotics were used to prevent illness and increase growth; however, nations worldwide are transitioning away from in-feed antibiotic administration due to antimicrobial resistance. Other strategies are being investigated to prevent PWD and maintain optimal growth performance. Studies suggest that providing LCP diet supplemented with CAA will yield similar results regarding the reproductive performance of sows and the growth performance of suckling pigs (Jang et al., 2014; Wang et al., 2014; Fang et al., 2019; Hu et al., 2023). It was speculated that feeding LCP diets to pigs would result in reduced reproductive performance and reduced growth in weaned pigs, however, supplementing CAA into LCP diets provides the animal with the 4 limiting AA needed for an ideal protein ratio (Wang et al., 2018). There is evidence that CAA supplementation may improve the performance of both sows and suckling pigs because intact protein cannot be completely released by digestive enzymes, whereas free form AA such as CAA are readily available for digestion and absorption, which can increase the digestibility of CP (Wang et al., 2018). The current study found that although HCP and LCP diets had different CP content, there were no significant differences in the AA composition between treatments, which can be attributed to CAA supplementation in the LCP diets.

### 3.6.1. SOW PERFORMANCE

#### BODY CONDITION

In the current study, CP content in the diet did not affect BW or BF in either gestation or lactation. Many studies have also shown that CP content in gestation and lactation diets does not affect sow BF or BF (Huang et al., 2013; Kim et al., 2023; Johannsen et al., 2024). Corley et al. (1983) reported that sows receiving LCP diets had achieved reductions in lactation weight loss during lactation when essential amino acids (EAA) were added compared to sows receiving LCP diets without EAA supplementation and HCP diets. The present study along with the others mentioned have supplemented LCP diets with EAA. By adding these specific AA, the diet can meet the sow's nutritional requirements more precisely, ensuring optimal performance and decreasing body condition lost during lactation (Wang et al., 2018). Therefore, LCP diets supplemented with EAA can be fed to sows without affecting the body condition during lactation.

Various studies report that sows receiving dietary PRO supplementation had less BW and BF loss during lactation compared to sows without PRO supplementation (Böhmer et al, 2006; Han et al., 2022; Konieczka et al., 2023). There is evidence to show that PRO reduce inflammation and improve the microbial environment in the gut (Cristofori et al., 2021). In addition, PRO can increase the intestinal surface area, increasing absorptive capacity of the gut, leading to improved nutrient absorption (Cristofori et al., 2021), which can help prevent body condition loss in the lactation period. In contrast, Khoudphaithoune et al. (2024) reported that BW and BF loss during lactation did not differ between sows receiving diets with *Bacillus subtilis* PRO supplementation and sows without PRO supplementation. The present study also found that BW did not differ throughout the entire experimental period based on PRO supplementation, however, BF loss tended to be lesser (*P* = 0.075) in sows supplemented with dietary PRO. Thus, PRO supplementation can reduce body condition loss in the lactation period.

## AVERAGE DAILY FEED INTAKE

Sow feed intake in the lactation period is important for overall productivity, longevity, and maintenance (Dourmad et al., 1994). Piglet growth and development is dependent on the sow diet and in order to support piglet growth, the sow needs to consume sufficient amounts of energy and nutrients. Lactating sows have high energy requirements to produce enough milk to feed the large number of piglets. Producing milk containing optimal EAA, vitamins and minerals is eminent to support the growth of piglets, and in order to do so, the sow needs to consume higher amounts of feed (Eissen et al., 2003). Traditionally, sows after farrowing are fed *ad libitum* to ensure they are provided enough nutrients to maintain their performance and support piglet growth (Eissen et al., 2003). In the current study, sows post-farrowing were provided with a 3 kg bag of feed which was increased stepwise until ad libitum was achieved. Weissensteiner et al. (2018) reported that sows receiving diets with 15% CP had decreased feed intake compared to sows receiving diets with 17.9% CP, to which the researchers attributed to the LCP diet containing both low CP and low lysine compared to the HCP diet and compared to previous recommended values. Many studies report that feeding sows LCP diets had no effect on lactation feed intake (Pedersen et al., 2019; Hu et al., 2023; Kim et al., 2023; Johannsen et al., 2024), which could be due to a variety of factors. Sows regulate their feed intake based on energy content of the diet rather than protein content alone, therefore even if the protein content of the diet is lower, if the energy content remains sufficient, reductions in feed intake may not be seen. In addition, sows prioritize nutrients for maintenance and lactation. If the LCP diet still provides sufficient AA for these critical functions, the sow may not reduce her feed intake. Many LCP diets are supplemented with EAA to ensure the sow has optimal amounts of AA to support her maintenance and her piglet's growth, therefore reductions in feed intake are not seen. The present study shows no differences between treatments for lactation feed intake, which agrees with previous literature.

As previously mentioned, sow feed intake during lactation is important for sow body condition, piglet weaning weights, return to estrus periods, and future productivity (Kiernan et al., 2023). Studies suggest that PRO can improve nutrient utilization by increasing contact with the lining of the gut, leading to increased absorption (Duttaroy et al., 2021), which can potentially lead to increased feed intake. Many studies have reported increases in ADFI in sows who received dietary PRO supplementation compared to control groups (Alexopoulos et al., 2003; Jeong et al., 2015; Kritas et al., 2015; Domingos et al., 2021). The inclusion of PRO in the sow diet can alter feed intake for a variety of reasons. As mentioned above, PRO increase the bioavailability of nutrients which leads to increased nutrient absorption (Butaye et al., 2003). By improving absorption, PRO ensure the sow receives more essential nutrients from the feed which can stimulate appetite and cause increased feed intake. Secretion of hormones involved in appetite regulation, such as ghrelin and leptin, can be influenced by PRO supplementation (Han et al., 2021). The supplementation of PRO may modulate the production of these hormones, promoting a more robust appetite in the sows (Han et al., 2021). In the current study, the supplementation of PRO did not affect ADFI of sows in lactation, which is not in accordance with other literature. The studies that showed increased feed intake when PRO was added to the diet used *Saccharomyces cerevisiae* (Domingos et al., 2021), *Bacillus subtilis* (Alexopoulos et al., 2003; Jeong et al., 2015; Kritas et al., 2015), or *Bacillus licheniformis* (Alexopoulos et al., 2003). In the present study, *Bacillus subtilis* and *Bacillus amyloliquefaciens* were used. Not all PRO strains have been shown to influence feed intake, therefore, the absence of increased feed intake in the current study may be due to the strains of PRO used (MacFarland et al., 2018). Moreover, the effectiveness of PRO can also depend on the existing microbial composition of the gut. In some cases, the microbiota way be well-balanced, therefore additional benefits such as increased feed intake will not be seen (Kothari et al., 2019).

## PLASMA UREA NITROGEN CONCENTRATION

Plasma urea nitrogen (PUN) has been used to determine requirements for protein and specific AA (Pedersen and Boisen, 2001). The value of PUN indicates the amount of urea circulating in the blood (Whang and Easter, 2000). Urea is an endogenous end product that is formed by the catabolism of AA that are not used in the body, therefore PUN provides an idea of how well protein is being digested and absorbed by the animal (Whang and Easter, 2000). Studies suggest that when CP content of the diet decreases, PUN decreases linearly (Nyachoti et al., 2006; Heo et al., 2009; Peng et al., 2016). This is because when protein is digested, it is broken down into nitrogen-containing compounds, and the compounds that are not utilized by the body are converted into urea. With a decreased intake of CP, there is less nitrogen available for conversion into urea. Consequently, the concentration of urea in the blood also decreases since there is less nitrogen being produced as a by-product of protein metabolism. In the present study, PUN concentrations were reduced in sows fed LCP diets on day 1 and day 20 of lactation, which agrees with previous findings.

Since PRO can improve the gut microbiota and the intestinal barrier, they can also increase digestion and absorption of nutrients, leading to decreased PUN values. Studies suggest that dietary PRO supplementation in the sow diet can result in decreased urea nitrogen concentrations (Gu et al., 2019; Zhu et al., 2022; Zhang et al., 2024). In the current study, the PUN levels of sows receiving PRO supplementation did not differ from sows receiving the control diet. Chen et al. (2006) also found that inclusion of dietary PRO did not affect PUN concentrations in finishing pigs. As previously mentioned, different strains of PRO yield different results, which may be why the PUN concentration was unaffected by the PRO in this study.

## FECAL DRY MATTER

Constipation during late gestation is common in sows due to restricted feeding programs. Feeding restricted diets in the late gestation period is a practice commonly used to avoid a high body condition score, which can cause prolonged parturition and lead to increases in stillborn piglets due to lack of oxygen (Mallmann et al., 2019). Many problems can arise when sows are constipated such as dystocia, uteritis and mastitis which can negatively influence the survival of piglets (Gu et al., 2019), which is why studies aim to investigate methods to prevent this from happening. In the current study, CP content and PRO supplementation in the sow diet did not influence fecal dry matter of sows. The study by Veleyudhan et al. (2018) reported similar results, where LCP diets containing 162 g/kg CP did not influence the apparent total tract digestibility of DM of sows compared to sows fed 179 g/kg CP. In contrast, Gomes et al. (2023) reported that feeding a LCP diet containing 13% CP to growing pigs increased fecal DM compared to pigs fed a diet containing 18.5% CP. Cristobal et al. (2023) reported similar results in which weanling pigs fed 17% CP had a higher amount of fecal DM than pigs fed diets containing 23% CP. Korniewicz et al. (2012) reported that sows receiving diets with a 20% reduction in CP had a numerically lower percentage of fecal DM compared to the control group.

Studies show differing effects of  PRO supplementation on fecal DM content. Jing et al. (2021) supplemented sow diets with *B. subtilis* and reported that fecal DM did not differ between sows with or without PRO supplementation. In addition, Sampath et al. (2024) reported that the fecal DM from weaned piglets born to sows supplemented with a mixture of *Bacillus subtilis* and B. *licheniformis* did not differ from piglets from sows without PRO supplementation. In contrast, Mazur-Kuśnirek et al. (2023) supplemented *Bacillus subtilis* and *B. amyloliquefaciens* to sows and weaned pigs which resulted in higher fecal DM in weaned pigs compared to pigs without PRO supplementation. Fecal DM may improve when PRO is supplemented due to several mechanisms. Probiotics can improve gut health by increasing the number of beneficial bacteria in the gut and therefore reduce the number of pathogenic bacteria that can cause diarrhea (Galli et al., 2024). In addition, a balanced microbiome can improve digestive efficiency, leading to increased digestion and the absorption of nutrients which can reduce the number of undigested materials being excreted which can lead to firmer feces (Kiernan et al., 2023). Moreover, intestinal mobility can be improved by PRO supplementation which ensures feed is moving through the GIT at an appropriate rate, preventing excess water secretion in the intestines which can affect fecal consistency (Galli et al., 2024).

### 3.6.2. REPRODUCTIVE PERFORMANCE

#### TOTAL BORN

Litter size of sows has been linearly increasing in recent years due to genetic improvement and sow management (Novak et al., 2020). The profitability of sow production is largely dependent on the reproductive potential of the sow, therefore increasing litter size has been made a priority. One method being investigated to increase litter size is feeding LCP diets to sows. There is evidence indicating that providing LCP diets to sows resulted in a higher number of total born piglets than sows receiving HCP diets (Jang et al., 2014; Fang et al., 2019). Diets containing HCP content have excess protein that the sow cannot digest, which leads to increased excretion and reduced feed efficiency. When feed efficiency is reduced, less nutrients are being absorbed and more are being excreted, which can reduce reproductive performance such as the total number of piglets born. However, it is possible that sows being fed LCP diets produce the same total number of piglets as sows fed HCP diets. When LCP diets are fed, they are often supplemented with EAA to ensure the sow is receiving all essential AA to meet their requirements for maintenance and gestation, therefore negative effects on reproductive performance will not be seen. In the current study, CP content of the sow diet did not influence the total number of piglets born, which has also been seen in previous studies (Falaschini et al., 1994; Huang et al., 2013; Kim et al., 2023).

Reproductive performance can be influenced by a variety of factors, such as genetics, herd management and nutrition (Ampode et al., 2023). The use of dietary PRO supplementation has been investigated to improve reproductive performance due to its effects on increasing feed efficiency and gut microbiota. Studies suggest that PRO supplementation into the sow diet increases the total number of piglets born per sow compared to sows without PRO supplementation. Saladrigas-Garćia et al. (2022) reported a significant increase in the total of number of piglets born from sows receiving *Bacillus amyloliquefaciens* PRO compared to sows without PRO supplementation. Wang et al. (2014) and Jeong et al. (2015) reported numerical differences in the total number of piglets born, where sows receiving diets supplemented with PRO had a higher number of piglets born compared to sows without PRO supplementation (*Lactobacillus johnsonii* and *Bacillus subtilis* + *Lactobacillus acidophilus* respectively). In the current study, the total number of piglets born did not differ between sows with or without PRO supplementation, which also agrees with previous literature by Hayakawa et al. (2016) and Khoudphaithoune et al. (2024). The role of PRO in the diet of sows is to improve gut health and feed efficiency, however, they do not directly influence reproductive performance, which may be the reason no increases in total number of piglets born were seen.

## LIVE BORN

Maintaining or increasing the number of live born piglets is of most importance since modern genetics and farm management have increased the total number of piglets per litter, which can lead to increased piglet mortality due to insufficient uterine space and nutrient supply, and increased parturition time (Langendijk and Plush, 2019). Feeding sows diets containing LCP has been investigated to study the effects of LCP diets on reproductive performance of sows. There are many studies suggesting that LCP sow diets do not influence the number of liveborn piglets (Gianluppi et al., 2020; Gregory et al., 2023; Kim et al., 2023; Johannsen et al., 2024). Since CP level did not impact number of liveborn piglets, this suggests that LCP diets can be fed to sows without negatively impacting reproductive performance. These results are also seen in the current study, as CP content did not have an effect on the number of piglets born alive.

In addition, PRO have been investigated on their effects of reproductive performance of sows. It is thought that PRO may improve reproductive performance as they aid in digestion and absorption of nutrients, leading to better nutrition for neonatal piglets. There are varying results in terms of the influence of PRO in the sow diet on the number of piglets born alive. For example, Böhmer et al. (2006), Baker et al. (2013), Zhang et al. (2020), and Nam et al. (2022) reported that supplementing PRO into the diet of sows increased the number of piglets born alive compared to sows fed diets without PRO supplementation (*Enterococcus faecium*, *Bacillus subtilis* + *B. amyloliquefaciens*, *B. subtilis*, and *B. subtilis* respectively). In contrast, Hayakawa et al. (2016) provided sow diets containing *B. mesentericus* PRO and reported no differences in the number of piglets born alive between sows with or without PRO supplementation. No differences between sows with or without PRO supplementation on the number of live born piglets were reported from Dominogos et al. (2021), in which *Saccharomyces cerevisiae* were supplemented. Similar results were presented by Konieczka et al. (2023) where diets were supplemented with a mixture of *Bacillus subtilis* and *B. amyloliquefaciens*. In the present study, there were no differences observed in the number of piglets born alive between sows with or without PRO supplementation, which agrees with several previous studies.

## STILLBORN

Due to the improvements in sow genetics, sows are now having larger litter sizes which can cause a higher rate of pre-weaning mortality. In sows with large litters, piglets may be in competition for space and nutrients in the uterus which can lead to inadequate development due to insufficient nutrient supply. In addition, large litter sizes are associated with an increased risk of dystocia and prolonged labour, which can lead to a higher number of stillborn piglets due to consecutive uterine contractions, reduced blood supply to the piglets, or decreased functionality of the umbilical cord (van den Bosch et al., 2022). In Canada, the average number of stillbirths was 1.05 and 1.19 in 2013 and 2023 respectively, with a steady increase across intervening years (PigChamp, 2024). Nutrition plays an important role in reproductive performance of sows, as nutritional deficiencies may cause increases in stillborn and mummified piglets as they may not receive proper nutrients to come to full term (Costa et al., 2019). To overcome this, LCP sow diets and PRO supplementation have been investigated.

There is evidence that feeding LCP diets to sows does not influence the number of stillborn piglets. Huang et al. (2013), Jang et al. (2014), and Kim et al. (2023) reported that the number of stillbirths did not differ between sows receiving LCP and HCP diets. In contrast, Weissensteiner et al. (2018) reported a numerical difference in the number of stillborn piglets, where sows receiving LCP diets had a reduced number of stillborn piglets than sows receiving HCP diets. In addition, Tydlitát et al. (2008) reported that sows receiving LCP diets had less stillborn piglets than sows receiving HCP diets. Johannsen et al. (2024) provided a low lysine and high lysine diet to sows in which the low lysine diet contained less CP than the high lysine diet. They reported that sows fed diets with lower amounts of lysine had a lower number of stillbirths than sows fed diets with high amounts of lysine The intestinal pH is reduced when LCP diets are fed because there is less undigested dietary protein (Luise et al., 2021), which leads to increased absorption of vitamins (Yamamura et al., 2023). This can lead to reductions in the number of stillborn piglets from sows receiving LCP diets, which the studies conducted by Weissensteiner et al. (2018) and Johannsen et al. (2024) suggest.

Nutrient absorption is crucial for reproductive performance of the sow. Increased nutrient absorption has been seen when PRO are supplemented into the sow diet. Some PRO strains produce enzymes that improve the bioavailability of minerals (Varvara and Vodnar, 2024), which can improve the absorption of these compounds. By increasing absorption, more nutrients are available to the sow for gestation, increasing the amount of nutrients for neonates, which can improve the reproductive performance of the sow. Khoudphaithoune et al. (2024) reported lower numbers of stillborn piglets in sows receiving diets supplemented with *Bacillus subtilis* PRO compared to sows without PRO supplementation. Nam et al. (2022) also reported that sows receiving supplementation of *Bacillus subtilis* had a reduced number of stillborn piglets compared to sows without supplementation. Although not statistically significant, Zhang et al. (2020) revealed that sows receiving supplementation of *Bacillus subtilis* had a numerically lower number of stillbirths compared to sows without supplementation. In contrast, Baker et al. (2013)

and Böhmer et al. (2006) reported that the number of stillborn piglets did not differ between the control and PRO group (*Bacillus subtilis* and *Enterococcus faecium* respectively).

In the current study, there was an interaction between LCP diets with PRO supplementation, where the number of stillborn piglets did not differ between the LCP and the LCP-PRO groups, while the number of stillborn piglets was lower in the HCP-PRO group compared to the HCP group. It has been reported than when birth weights are increased, stillbirths are decreased (Baxter et al., 2008; Baxter et al., 2009; Nam et al., 2020; Nam and Sukon, 2021). As seen above, piglets from sows receiving LCP diets had increased birth weights compared to piglets from sows receiving HCP diets, which suggests the interaction effect of LCP and PRO on the number of stillbirths may be correlated with increased birth weights.

## MUMMIFIED

As with stillbirths, the average number of mummified piglets has been increasing within recent years. As the average number of piglets per litter increases, the average number of mummified piglets also increases, which can be caused by insufficient nutrients being provided to each fetus (van den Bosch et al., 2022). The average number of mummified piglets in Canada is 0.35 and 0.44 for 2013 and 2023, respectively, with steady increases within years (PigChamp, 2024). These steady increases are alarming and why methods to decrease the number of mummified piglets is being investigated. Several studies reported that feeding diets with LCP to sows did not affect the number of mummified piglets compared to sows fed HCP diets (Velayudhan et al., 2018; Weissensteiner et al., 2018; Gregory et al., 2023; Kim et al., 2023). Although the number of mummified piglets did not decrease, maintaining the number of mummified piglets seen when sows are fed HCP diets when providing LCP diets is still beneficial. In order for fetuses to develop, sufficient amounts of protein must be provided in the sow diet. It could be speculated that decreases in dietary CP content could cause increases in the number of mummified piglets as the sow is receiving less nutrients which could cause decreases in the amount of nutrients the fetuses are receiving. However, LCP diets are often supplemented with EAA to ensure the sow is provided optimal nutrition to maintain performance, therefore decreases in reproductive performance, such as increases in the number of mummified piglets, are not seen.

During the late stages in gestation, sows can start suffering from oxidative stress, which is a process that involves an accumulation of active oxygen particles produced by the placenta and mammary glands (Li et al., 2022). Oxidative stress can cause sows to reduce feed intake and can impact proper fetal development, which can result in a higher number of stillborn and mummified piglets (Li et al., 2022). There is evidence that suggests supplementing PRO in the sow diet can reduce oxidative stress, thus improve reproductive performance (Shen et al., 2011; Cui et al., 2019; Sun et al., 2020; Sun et al., 2021). Supplementing PRO in the sow diet can also aid in digestion and absorption of nutrients since they improve the gut microbiome and increase surface area in the intestinal tract, allowing for higher absorptive capacity of the gut. Alexopoulos et al. (2003) and Nam et al. (2022) reported that providing diets to sows supplemented with PRO decreased the number of mummified piglets compared to sows receiving diets without PRO supplementation (*Bacillus licheniformis* + *B. subtilis* and *Bacillus subtilis* respectively). In contrast, Lipiński et al. (2012), Wang et al. (2014), Saladrigas-Garćia et al. (2022) and Sampath et al. (2024) reported no differences in the number of mummified piglets between sows fed with or without PRO (*Saccharomyces cerevisiae, Lactobacillus johnsonnii, Bacillus subtilis* + *B. Licheniformis*, and *B. subtilis* + *B. licheniformis* respectively).

In the current study, there was an interaction effect between CP content and PRO supplementation where the number of mummified piglets did not differ between HCP and HCP-PRO groups, but the number of mummified piglets was lower in LCP-PRO compared to LCP. This result shows that the synergistic effects of LCP sow diets with PRO supplementation can reduce the number of mummified piglets being born and improve reproductive performance of sows.

### 3.6.3. COLOSTRUM AND MILK COMPOSITION

Ensuring each piglet consumes an adequate quantity and quality of colostrum is vital in maintaining the viability of each piglet. Colostrum is the main source of energy for newborn piglets, and it contains essential nutrients as well as maternal antibodies that contribute to passive immunity (Blavi et al., 2021). The nutrients consumed by the sow have a great influence on the quality of colostrum and milk provided to her piglets, therefore, providing the optimal amount of AA to the sows can improve potential performance of her piglets. A study conducted by Hojgaard et al. (2019) reported that the fat and lactose content of milk did not differ among sows fed LCP diets supplemented with EAA and sows fed HCP diets. In addition, Zhang et al. (2019) fed diets containing 14.25% CP with supplementation of EAA and reported the fat, lactose and protein content of the milk did not differ amongst sows fed HCP (17.25% CP) and LCP diets. In the current study, neither CP content or PRO supplementation into the sow diet had an influence on the fat, protein, or lactose content of either colostrum or milk, which agrees with previous studies.

### 3.6.4. PIGLET PERFORMANCE

### BODY WEIGHT

The variation in sow litters has grown due to the genetic advances in sow litter size. Smaller piglets at birth are less likely to survive until weaning because the large piglets will take precedence at the teat, leaving the small, weak piglets without sufficient amounts of colostrum and milk which is vital to their growth and development (Geiping et al., 2022). High litter weights are important in maintaining the viability of the piglets after birth. There is evidence to suggest that feeding sows diets containing LCP can increase litter weights of their piglets. Hu et al. (2023) fed sows diets containing 14.5% CP and found that piglets from sows receiving the LCP diet had higher average weaning weights compared to piglets from sows fed a diet containing 17.5% CP. Fang et al. (2019) also found that piglets from sows receiving a diet containing 10.5% CP had increased weaning weights compared to piglets from sows fed diets containing 13.5% CP. In comparison, Jang et al. (2018), Gregory et al. (2023), and Kim et al. (2023) reported no differences in piglet or litter weights from sows fed LCP diets compared to piglets from sows fed HCP diets (11% vs 17%, 15.9% vs 17.9%, and 11% vs 16% respectively).

There is a wide array of research that shows PRO supplementation in the sow diet can increase piglet and litter weights, as well as increase piglet ADG. Hayakawa et al. (2016) supplemented a multi-strain PRO containing *Bacillus mesentericus*, *Clostridium butyricum* and *Enterococcus faecalis* into the sow diet, which resulted in increased piglet birth weights compared to piglets from sows without PRO supplementation, however, there was no difference in piglet weights on day 7 post-farrowing. Zhang et al. (2020) supplemented sow diets with *Bacillus subtilis* and reported that sows provided diets with PRO supplementation had higher litter weaning weights compared to sows without PRO supplementation. Nam et al. (2022) also supplemented *B. subtilis* PRO to sows and reported higher litter birth weight and litter weights in the overall experimental period compared to litters from sows without PRO supplementation. Similar to the current study, Konieczka et al. (2023) provided a combination of *Bacillus subtilis* and *B. amyloliquefaciens* PRO in the sow diet, which resulted in increased litter weight at weaning and overall total weight gain compared to litters from sows without PRO supplementation. Moreover, Sampath et al. (2024) supplemented a mixture of *Bacillus subtilis* and *B. licheniformis* to sows which resulted in increased ADG during *E. coli* challenge compared to piglets from sows without PRO supplementation. Zhu et al. (2023) supplemented a mixture of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* PRO into the sow diet, which resulted in increased ADG at 125 days of age compared to pigs from sows not receiving PRO supplemented diets. In the current study, piglets from sows fed LCP diets had heavier birth weight compared to piglets from sows receiving the HCP diet, however, piglets from sows receiving HCP diets had lower ADG during week 1 compared to piglets from sows receiving LCP diets. There was no effect of CP or PRO on BW on day 7 or 14 post-farrowing, however there was a tendency for increased BW in piglets from sows fed HCP diets compared to piglets from sows fed LCP diets.

## PLASMA UREA NITROGEN CONCENTRATION

As previously mentioned, PUN is an indicator of protein and AA utilization in pigs. A high PUN concentration is indicative of high amount of undigested and not synthesized protein in the blood (Marín-García et al., 2022). Piglets are less efficient at digesting nutrients as they possess an immature digestive system and the gut microbiome is not fully developed, which can lead to increased PUN concentrations. Providing LCP diets to sows is thought to decrease PUN concentrations in both sows and suckling pigs. Since less protein is being supplied, the amount of excess nutrients will decrease and allow for the majority of the protein supplied to be digested and absorbed by the body. Heo et al. (2008) found that weaned pigs receiving diets containing 173 g CP/kg had decreased PUN concentrations compared to pigs receiving 243 g CP/kg. Zervas & Zijlstra (2002a) reported that growing pigs receiving 15.5% CP had reduced PUN concentrations than pigs receiving 18.5% CP. Another study by these authors showed similar results, where growing pigs fed diets with 13.6% CP had lower PUN concentrations than pigs fed 16.5% CP (Zeras & Zijlstra, 2002b). In contrast, Kroeske et al. (2021) found that piglets from sows receiving LCP diet had increased PUN levels at weaning compared to piglets from sows receiving HCP diets, however they stated that the reason for this result is unclear, thus the experiment should be repeated to confirm results found. There is evidence that PRO supplementation can decrease PUN levels as they can improve digestion by regulating the balance of intestinal microbiome by outcompeting pathogenic bacteria, allowing beneficial bacteria to proliferate (Zhang et al., 2023). This has been shown in a study by Link & Novak (2006), where weaned pigs supplemented with *Bacillus subtilis* and *B. licheniformis* showed decreased PUN levels on day 70 of age compared to pigs without PRO supplementation.

## FECAL DRY MATTER

Piglets are more susceptible to diarrhea which can lead to loss of production and death. The intestinal barrier function is impaired which increases permeability, allowing for bacteria to proliferate and spread throughout the body. The gut of piglets is rapidly colonized by bacteria and with an immature immune system, it can be more difficult for these pigs to fight diseases which can led to diarrhea. Diarrhea can cause dehydration, lethargy and reduced feed intake which will decrease the growth performance. Methods to improve fecal DM of piglets is of most importance to reduce loss of production. Feeding sows LCP diets has been shown to improve fecal DM of piglets. Heo et al. (2008) reported that weaned pigs fed LCP diets (173 g CP//kg) had higher fecal DM compared to pigs receiving HCP diet (243 g CP/kg). Marchetti et al. (2023) indicated that weaned pigs fed a diet containing 15.5% CP had less incidence of diarrhea compared to pigs fed diets containing 17.5% CP, which is indicative of piglets fed LCP diets having a higher fecal DM percentage. Kroeske et al. (2021) also reported that feeding LCP (122 g/kg) diets to sows resulted in higher piglet fecal DM on day 7 and day 10 post-farrowing compared to piglets from sows receiving HCP (168 g/kg) diets. On the contrary, Bellego & Noblet (2002) reported that there were no differences in piglet fecal DM between pigs receiving 169, 184, 204, or 224 g CP/kg. Fecal consistency can be improved by feeding LCP diets because LCP diets reduce protein fermentation in the large intestine, reducing the production of toxic by-products such as ammonia that can irritate the gut resulting in increased incidence of diarrhea (Pluske et al., 2002).

There are mixed results when using PRO to increase fecal DM of piglets and weaned pigs. Mazur-Kuśnirek et al. (2023) found that supplementing *Bacillus subtilis* and *B. amyloliquefaciens* to weaned pigs resulted in higher fecal DM percentages that weaned pigs without PRO supplementation. However, Biswas et al. (2023) reported that weaned pigs supplemented with a mixture of *B. Subtilis, B. licheniformis*, *B. coagulans*, and *Clostridium butyricum* had similar fecal DM to pigs without PRO supplementation. In addition to this, Jing et al. (2021) reported that piglets from sows fed diets containing *Bacillus subtilis* and *B. licheniformis* had similar fecal DM to piglets from sows without PRO supplementation.

In the present study, there was an interaction between CP and PRO on day 21 piglet fecal DM, where there were significant differences found between HCP and HCP-PRO groups, however, LCP and LCP-PRO groups did not differ. On day 28, fecal DM was lower in piglets from sows receiving diets supplemented with PRO compared to piglets from sows who did not receive PRO supplementation. Mazur-Kuśnirek et al. (2023) used the same mixture of PRO for their study as was used for this study, which suggests the combination of *B. subtilis* and *B. amyloliquefaciens* can improve piglet fecal DM.

### 3.6.5. INDICES OF GUT HEALTH

Gut health plays a crucial role in many aspects of a pig's life, including immune function, disease resistance, gut barrier function, digestion, and production efficiency (Guevarra et al., 2019). Immediately after birth, piglets are introduced to bacterial populations from colostrum, and from external factors such as sow feces (Luo et al., 2022b). Since piglet gut and immune function are not full developed at this stage, contact with harmful bacteria can cause reduction is production potential due to illness or death. As mentioned previously, diarrhea-causing bacteria such as *E.coli* and *Clostridium* can be lethal to young pigs, therefore, methods to prevent adhesion of these pathogens in the gut are being investigated. Gut health is also important for sows since sows transfer maternal beneficial microbes to suckling pigs. Therefore, the healthy gut of sows is important in ensuring the piglets receive proper antibodies to increase their vitality post weaning. In the current study, there was an interaction effect of CP and PRO on d 21 piglet relative abundance of *E.coli*, where the abundance of *E.coli* was lower in piglets from sows fed HCP-PRO diets compared to piglets from sows fed HCP diets. In contrast, piglets from sows fed LCP diets had a lower abundance of *E.coli* than piglets from sows receiving LCP-PRO diets on d 21 post-farrowing. On d 28, piglets from sows receiving LCP diets had a decreased fecal abundance of *E.coli* compared to piglets from sows fed HCP diets. Wellock et al. (2008) reported similar results where weaned pigs fed 130 g/kg CP had reduced *E.coli* counts in the jejunum compared to pigs fed 230 g/kg CP. Opapeju et al. (2015) found that weaned pigs fed diets containing 17.3% CP had reduced jejunal *E.coli* counts compared to pigs receiving diets with 22.2% CP. A decreased relative abundance of *E. coli* was seen in weaned pigs receiving LCP diets compared to pigs receiving HCP diets in a study conducted by Pollock et al. (2019), which agrees with the current findings. Many studies suggest that PRO supplementation can reduce the relative abundance of *E.coli* in pigs. Daudelin et al. (2011) reported that weaned pigs had decreased *E. coli* attachment in the ileum mucosa when fed diets containing *Pediococcus acidilactici* and *Saccharomyces cerevisiae boulardii* PRO compared to pigs without PRO supplementation. Lee et al. (2012) reported similar results, where groups of weaned pigs supplemented with *Lactobacillus plantarum* had less pigs positive for *E.coli* infection compared to pigs without PRO supplementation. Pan et al. (2017) took cecal samples from weaned pigs fed diets supplemented with *Bacillus licheniformis* and *Saccharomyces cerevisiae* and reported that pigs supplemented with the PRO mixture had decreased cecal *E. coli* counts compared to those without PRO supplementation. This shows that multiple strains of PRO have the potential to reduce the abundance of *E. coli* in the GIT of piglets and weaned pigs.

*Clostridium spp*. bacteria can have negative impacts on swine production. A specific strain called *Clostridium perfringens* is a gram-positive bacterium that can cause hemorrhagic diarrhea in pigs which can lead to lethargy and death (Burrough, 2021); thus this bacterium can result in high losses of production therefore methods to prevent the risk of *Clostridium perfringens* is being investigated. There is evidence to suggest that LCP diets and PRO supplementation can prevent *Clostridium* abundance in the GIT of pigs. Fan et al. (2017) and Liao et al. (2024) showed that there was a decreased abundance of *Clostridium* bacteria in the ileum of pigs receiving LCP diets compared to pigs receiving HCP diets. In addition, Wang et al.

(2023) reported reduced counts of *Clostridium* bacteria in the GIT of pigs fed diets with 12% CP compared to pigs fed diets with 14-16% CP. Vasquez et al. (2023) reported that PRO supplementation may also reduce *Clostridium* counts in the GIT of pigs. These researchers supplemented a multispecies PRO containing *Bacillus amyloliquefaciens, Levilactobacills brevis, Bacillus subtilis* and *Limosilactobacillus reuteri* and found that both HCP-PRO and LCP-PRO groups had reduced counts of *Clostridium* in the GIT compared to pigs without PRO supplementation. Cho et al. (2024) conducted a study with weaning to finishing pigs which found that pigs receiving PRO supplementation had decreased *Clostridium* counts in the ileum compared to pigs without PRO supplementation, although the strain of PRO used were not specified. At 10 weeks of age, Park et al. (2024) reported that pigs supplemented with a mixture of *Enterococcus faecium*, *Bacillus subtilis* and *Saccharomyces cerevisiae* had decreased *Clostridium* counts compared to pigs without PRO supplementation. In the current study, there were no differences seen between treatment groups on d 21 *Clostridium perfringens* counts. In contrast, there was an interaction effect of CP content and PRO supplementation on d 28 post farrowing where pigs from sows receiving HCP diets had a decreased relative abundance of *Clostridium perfringens* compared to sows receiving HCP-PRO diet, yet there were no differences between LCP and LCP-PRO groups. This shows that there is the potential for PRO to reduce the incidence of PWD in weaned pigs.

Achieving microbial balance in the GIT is vital for efficient pig production. Beneficial bacteria need to outweigh the presence of harmful bacteria to establish a healthy GIT. One such bacteria that can improve overall production of pigs is *Lactobacillus* spp. *Lactobacillus* can improve gut health in both sows and piglets. It has been shown to improve intestinal health by modulating gut bacteria leading to reduced risk of infection such as PWD. They modulate the gut microbiome by competing with harmful bacteria for attachment sites in the gut lining, which can reduce the colonization of harmful bacteria that can lead to illness. *Lactobacillus spp*. can stimulate immune function in the GIT, increasing the production of antibodies and other immune factors, which can help protect both sows and piglets from disease. Through improved digestion and absorption, *Lactobacillus* bacteria can improve production efficiency by ensuring high conversion efficiency allowing for better utilization of nutrients for growth and production, therefore, methods to increase the abundance of *Lactobacillus* in the GIT of pigs are being investigated. Gao et al. (2022) reported that feeding diets containing 17% casein protein resulted in increased *Lactobacillus* counts in weaned pigs compared to diets with 22% protein. Mi et al. (2023) reported that feeding diets containing 14% CP resulted in increased *Lactobacillus* counts in the jejunum of weaned pigs compared to pigs fed diets containing 17% CP. According to a study conducted by Liu et al. (2024), pigs receiving diets with 16% CP had increased *Lactobacillus* counts in the jejunum at 5-10 weeks of age compared to pigs fed diets containing 16, 18, and 22% CP at the same growth stage. Probiotics have also been said to increase the presence of beneficial bacteria such as *Lactobacillus*, which is possible as probiotics produce antimicrobial agents that can supress the growth of harmful bacteria (Hemarajata and Versalovic, 2013). This has been shown in multiple studies such as one conducted by Amhed et al. (2014) in which feeding weaned pigs a diet supplemented with a multispecies PRO containing *Lactobacillus reuteri, Bacillus subtilis* and *Bacillus licheniformis* resulted in increased *Lactobacillus* counts in feces on d 7 and 14 compared to pigs fed the control diet. Lan et al. (2016b) provided a multispecies PRO containing *Bacillus subtilis, Bacillus coagulance, Bacillus licheniformis*, and *Clostridium butyricum* to weaned pigs which resulted in increased fecal *Lactobacillus* counts compared to those fed a control diet. Another study conducted by Zhou et al. (2021) reported that sows fed *Bacillus subtilis* and *Enterococcus faecium* fermented feed had an increased relative abundance of *Lactobacillus* in feces on d 7 and 14 compared to sows fed control diets. In the current study, there was an interaction effect between CP content and PRO supplementation on the relative abundance of *Lactobacillus* bacteria in piglets on d 21 post-farrowing, where piglets from sows receiving HCP diets had a lower relative abundance of *Lactobacillus* compared to piglets from sows receiving HCP-PRO diets, however, piglets from sows fed LCP-PRO diets had a lower relative abundance of *Lactobacillus* compared to piglets from sows fed LCP diets. In addition, sows receiving PRO-supplemented diets tended to have a higher relative abundance of *Lactobacillus* in the gut compared to sows without PRO supplementation, which agrees with previous findings.

Another beneficial bacterium in the gut of sows is *Bifidobacterium*. There are many positive effects of *Bifidobacterium* in the gut such as improving intestinal development, improving antioxidant capacity, and balancing microbiota in the gut (Pang et al., 2022). By improving the fermentation of non-digestible carbohydrates into SCFAs, they improve overall digestive health to support efficient nutrient absorption which can improve sow performance (Galli et al., 2024). Gao et al. (2022) reported that LCP diets can increase the abundance of Bifidobacterium in weaned pigs. In this study, weaned pigs receiving diets containing 17% CP had increased Bifidobacterium counts in the GIT compared to pigs fed diets containing 30% CP. Liu et al. (2016) showed similar results with 20-30kg pigs having a higher abundance of *Bifidobacterium* when fed 10% CP compared to pigs fed 12, 14, 16, and 18% CP diets. Zhang et al. (2020b) fed LCP diets to weaned pigs. From d 42-77, pigs were fed a diet containing either 16% or 20% CP, and reported that the pigs fed 16% CP had higher counts of *Bifidobacterium* in the ileum and jejunum than pigs fed diets with 20% CP. The same results were reported from d

77-120, where pigs fed diets containing 14% CP had increased counts of *Bifidobacterium* than pigs fed 18% CP. In contrast, Rist et al. (2017) and Kaewtapee et al. (2017) found that pigs fed HCP diets had an increased number of *Bifidobacterium* in the gut compared to pigs fed LCP diets. Kaewtapee et al. (2017) stated the reason for this difference was due to the high SBM content of the HCP diet which increased the availability of fermentable carbohydrates, stimulating the growth of *Bifidobacterium* in the ileum. In the present study, there was no effect of CP content on the relative abundance of *Bifidobacterium* in feces of sows. Bifidobacterium is not considered a dominant group in the swine intestine. In a study conducted by Loh et al. (2006), only 40% of sows had Bifidobacterium detected in digesta samples. Since the prevalence of *Bifidobacterium* in the swine gut is scarce,  the small number of *Bifidobacterium* in all groups may be why no differences were seen between treatment groups in the present study.

The abundance *Bifidobacterium* has been said to increase when PRO is added to the diet of pigs. This is due to several mechanisms, all of which improve gut health. There is evidence that PRO can outcompete harmful bacteria which allows for improved growth of Bifidobacterium. Improvements in gut barrier function and modulation of immune response are also effects of PRO supplementation which allow pigs to maintain balanced gut microbiome. Modesto et al. (2009) reported that weaned pigs receiving diets supplemented with *Bifidobacterium anamalis* had increased *Bifidobacterium* counts in caecum contents compared to other treatment groups. Pang et al. (2022) also fed diets containing *Bifidobacterium anamalis* to weaned pigs and reported that pigs with PRO supplementation tended to have higher counts of *Bifidobacterium* in ileal digesta compared to pigs without on a control diet. In the current study, there was no effect of PRO supplementation on the relative abundance of *Bifidobacterium* in fecal samples from sows. As previously stated, the abundance of *Bifidobacterium* in the swine gut is low, therefore, differences between treatments may not be seen.

## 3.7 CONCLUSION

In conclusion, feeding LCP diets with PRO supplementation to sows resulted in improved reproductive performance and improved gut health of suckling pigs. This is shown in the number of stillborn and mummified piglets where sows receiving diets supplemented with PRO had a lower number of piglets born stillborn (HCP-PRO) and mummified (LCP-PRO) compared to those fed diets without PRO supplementation. Feeding LCP-PRO diets to sows has the potential to reduce the risk of PWD. Piglets from sows fed HCP-PRO diets had a higher percentage of fecal DM compared to the HCP group on day 21, and piglets from sows receiving PRO-supplemented diets had a higher percentage of fecal DM on day 28 compared to piglets from sows without PRO-supplemented diets. Providing sows with LCP diets did not affect the growth performance of piglets which shows that although less protein is being provided, high production efficiency can be achieved.

# CHAPTER 4: GENERAL DISCUSSION & CONCLUSION

## 4.1. GENERAL DISCUSSION

Weaning is a stressful time for pigs as they are exposed to a variety of new stressors such as change in feed, environment, and social structures (Tang et al., 2022c). Weaning stress can cause changes in intestinal morphology, disrupts digestion and absorption of nutrients, alters intestinal barrier function, and can lead to increased incidence of diarrhea and reductions in growth (Tang et al., 2022a). Due to immature immune and digestive systems, weaned pigs are most susceptible to disease, such as PWD, which can cause dehydration, morbidity, and mortality (Rhouma et al., 2017). Various methods have been tested to mitigate the risk of PWD infection in swine herds such as in-feed antibiotics, vitamin supplementation, and prebiotic supplementation. In recent years, the use of LCP diets and PRO supplementation has been investigated as a strategy to prevent PWD infection, however, these diets are provided directly to weaning pigs and are provided separately. In the present study, LCP diets with PRO supplementation were provided to sows to assess the synergistic effects on reproductive performance and piglet performance and gut health.

In the current study, there was an interaction effect of CP and PRO supplementation on the number of stillborn piglets where the number of stillborn piglets did not differ between the LCP and the LCP-PRO group, while the number of stillborn piglets was lower in HCP-PRO group compared to the HCP group. Moreover, there was an interaction effect of CP and PRO on the number of mummified piglets where the number of mummified piglets did not differ between HCP and HCP-PRO groups, but the number of mummified piglets was lower in LCP-PRO compared to LCP. This shows that PRO supplementation can improve reproductive performance of sows. Khoudphaithoune et al. (2024) reported lower numbers of stillborn piglets in sows receiving diets supplemented with *Bacillus subtilis* PRO compared to sows without PRO supplementation. In addition, Nam et al. (2022) reported that sows receiving supplementation of *Bacillus subtilis* had a reduced number of stillborn piglets compared to sows without supplementation. Reductions in the number of mummified piglets were reported by Alexopoulos et al. (2003) and Nam et al. (2022). They showed that providing diets to sows supplemented with PRO decreased the number of mummified piglets compared to sows receiving diets without PRO supplementation (*Bacillus licheniformis* + B. *subtilis,* and *Bacillus subtilis* respectively), which agrees with the results of the current study.

The concentration of PUN in the blood is a direct indicator of AA breakdown (Coma et al., 1996). A low PUN concentration is indicative of more efficient protein and AA breakdown. Feeding HCP diets to pigs can lead to higher PUN concentrations because they increase the intake of nitrogen, which is metabolized into urea for excretion. Converting nitrogen into urea is a process that uses an extensive amount of energy, which can cause depleted energy for pregnancy and milk production in sows, and growth in piglets (Kohn et al., 2005). In the current study, there was an effect of CP on sow PUN concentrations on d 1 and d 20 of lactation, where sows fed LCP diets had lower PUN concentrations than sows receiving HCP diets. Studies have shown that as dietary CP decreases, PUN concentrations decrease linearly. Nyachoti et al. (2006) reported that PUN decreased linearly as dietary CP was reduced from 23% to 19% and 21%. Heo et al. (2009) fed diets containing 243 g/kg CP and 173 g/kg CP and found that pigs fed LCP diets had lower PUN values than pigs fed HCP diets. Peng et al. (2016) also reported that PUN values decreased linearly in pigs fed 20, 17.16, 15.3 and 13.4% CP. There was no effect of PRO supplementation on sow PUN concentrations.

Days before parturition, sows are often provided a restricted feed allowance in order to decrease soluble fibre intake. Soluble fibre is more fermentable and can cause digestive issues if consumed in excess (Oh et al., 2024). Moreover, increased fibre intake during this stage can cause constipation which can cause prolonged farrowing and can lead to an increased stillborn rate (Tabeling et al., 2003). Following constipation, the release and absorption of endotoxins is increased which can cause the development of mastitis (Tabeling et al., 2003); therefore reducing the risk of constipation is important for sow performance. In the current study, sow fecal DM did not statistically differ between treatments. This shows that feeding LCP to sows does not increase fecal DM and thus the risk of constipation is not increased. Similar results have been reported by Veleyudhan et al. (2018) where LCP diets containing 162 g/kg CP did not influence apparent total tract digestibility of DM of sows compared to sows fed 179 g/kg CP. In addition, Han et al. (2023) reported no differences between weaned pigs fed diets containing 23.7% and 18.9% CP. It is thought that supplementing PRO to pigs can improve fecal DM as they increase absorption of nutrients and can improve the gut microbiome. Jing et al. (2021) and Sampath et al. (2024) provided diets with *Bacillus subtilis* PRO to pigs, however, there was no difference in fecal DM content between pigs with and without PRO supplementation, which agrees with the results of the current study.

In swine production, maintaining a healthy GIT is very important. For this to occur, there must be more beneficial bacteria in the gut than pathogenic bacteria. One such healthy bacteria is *Lactobacillus*. This bacterium can help regulate the immune system and balance the microbiome in the gut (Valeriano et al., 2017). There is evidence to show that LCP diets and PRO supplementation can improve relative abundances of *Lactobacillus* bacteria in the GIT. Gao et al. (2022) reported that HCP diets led to decreased counts of *Lactobacillus* in the gut than LCP diets in weaned pigs. Moreover, Mi et al. (2023) reported that weaned pigs receiving diets with 14% CP had higher numbers of *Lactobacillus* in the jejunum than pigs fed a diet consisting of 17% CP. In the current study, there were no differences seen between treatments due to protein content of the diet, however, sows receiving PRO-supplemented diets tended to have a higher relative abundance of *Lactobacillus* in the gut compared to sows without PRO supplementation. Previous studies have come to the same conclusion. Lan et al. (2016b) provided a multispecies PRO containing *Bacillus subtilis, Bacillus coagulance, Bacillus licheniformis*, and *Clostridium butyricum* to weaned pigs which resulted in increased fecal *Lactobacillus* counts compared to those fed a control diet. Zhou et al. (2021) also reported that sows fed *Bacillus subtilis* and *Enterococcus faecium* fermented feed had an increased relative abundance of *Lactobacillus* in feces on d 7 and 14 compared to sows fed control diets which agrees with the results of the present study.

The birth weight of piglets can be an indicator of their viability once weaned. As litter size increases, the number of piglets born per litter increases which can cause a high proportion of small piglets (Fix et al., 2010). Thus, strategies to increase piglet birth weight are being investigated. In the current study, piglets born from sows receiving LCP diets had higher birth weights than piglets from sows fed HCP diets. Similar results have been shown in the past by Kim et al. (2021) and Gregory et al. (2023). These studies report that sows receiving diets with 17% CP did not negatively affect piglet birth weights compared to sows receiving diets containing 19% CP. In addition, Mejia-Guadarrama et al. (2002) and Pike & Boaz. (2009) reported than feeding diets with 10% CP also did not negatively affect piglet birth weight or ADG of piglets compared to piglets from sows receiving diets with 20% and 19% CP respectively, which agrees with the findings in this study. There was no effect of PRO supplementation on piglet BW, however, many studies show that PRO supplementation can improve piglet BW. Zhang et al. (2020) supplemented sow diets with *Bacillus subtilis* and reported that sows receiving PRO supplemented diets had higher litter weaning weights compared to sows without PRO supplementation. Nam et al. (2022) also supplemented *B. subtilis* PRO to sows and reported higher litter birth weight and litter weights in the overall experimental period compared to litters from sows without PRO supplementation.

Methods to increase piglet fecal DM have been studied extensively as a major loss of production is diarrhea-causing diseases which can lead to morbidity and mortality. A major source of loss of production is PWD, which is characterized by severe diarrhea, dehydration, lethargy, and death (Rhouma et al., 2017). One strategy thought to decrease the risk of PWD is feeding LCP diets and supplementation of PRO. Heo et al. (2008) reported that weaned pigs fed 173 g CP//kg had higher fecal DM compared to pigs receiving 243 g CP/kg. Marchetti et al. (2023) reported that weaned pigs fed a diet with 15.5% CP had less incidence of diarrhea compared to pigs fed diets with 17.5% CP, which is indicative of piglets fed LCP diets having a higher fecal DM percentage. Kroeske et al. (2021) also reported that feeding 122 g/kg CP diets to sows resulted in higher piglet fecal DM on day 7 and day 10 post-farrowing compared to piglets from sows receiving 168 g/kg CP diets. Mazur-Kuśnirek et al. (2023) found that supplementing *Bacillus subtilis* and *B. amyloliquefaciens* to weaned pigs resulted in higher fecal DM percentages that weaned pigs without PRO supplementation. However, Biswas et al. (2023) reported that weaned pigs supplemented with a mixture of *B. Subtilis, B. licheniformis*, *B. coagulans*, and *Clostridium butyricum* had similar fecal DM to pigs without PRO supplementation. In the present study, there was an interaction between CP and PRO on day 21 piglet fecal DM, where there were significant differences found between HCP and HCP-PRO groups, however, LCP and LCP-PRO groups did not differ. On day 28, fecal DM was lower in piglets from sows receiving diets supplemented with PRO compared to piglets from sows who did not receive PRO supplementation, which is in accordance with previous findings.

Piglets are more susceptible to disease and due to their immature immune systems, can cause severe illness and death. As previously mentioned, one of the deadliest illnesses among piglets is PWD because of its ability to cause severe loss of appetite and dehydration which in most cases leads to death (Rhouma et al., 2017). Enterotoxigenic *Escherichia coli* is one of the most common pathogens that cause diarrheal diseases such as PWD, therefore, maintaining the microbial environment of the gut is necessary in preventing ETEC adhesion to the gut and thus preventing PWD infection. Studies show that lowering the CP content of the diet can have beneficial effects on the microbial communities in the gut of pigs. Wellock et al. (2008) reported that weaned pigs fed 130 g/kg CP had reduced *E.coli* counts in the jejunum compared to pigs fed 230 g/kg CP. In addition, Opapeju et al. (2015) states that weaned pigs fed diets containing 17.3% CP had reduced jejunal *E.coli* counts compared to pigs receiving diets with 22.2% CP. A decreased relative abundance of *E. coli* was seen in weaned pigs receiving LCP diets compared to pigs receiving HCP diets in a study conducted by Pollock et al. (2019). In the current study, on there was an interaction effect of CP and PRO on d 21, where the abundance of *E.coli* was lower in piglets from sows fed HCP-PRO diets compared to piglets from sows fed HCP diets, however, piglets from sows fed LCP diet had a lower abundance of *E.coli* than piglets from sows receiving LCP-PRO diets. On d 28, pigs from sows fed LCP diets had a decreased relative abundance of *E.coli* compared to pigs from sows fed HCP diets. In addition, there was an interaction effect of CP and PRO on d 21 on the relative abundance of Lactobacillus, where piglets from sows receiving HCP diets had a lower relative abundance of *Lactobacillus* compared to piglets from sows receiving HCP-PRO diets, however, piglets from sows fed LCP-PRO diets had a lower relative abundance of *Lactobacillus* compared to piglets from sows fed LCP diets. A similar effect was reported in the relative abundance of *Clostridium perfringens* on d 28, where pigs from sows receiving HCP diets had a decreased relative abundance of *Clostridium perfringens* compared to sows receiving HCP-PRO diets.

## 4.2. GENERAL CONCLUSION

The prevalence of PWD in swine herds can cause extreme losses in production and reduce production efficiency. Illness in weaned pigs may be caused by insufficient nutrition or insufficient antibodies to fight illness which can result in death. In-feed antibiotics have been banned which leaves producers struggling without alternative to prevent and address illness in weaned pigs. Proper sow nutrition is vital in ensuring piglets are receiving adequate nutrients and antibodies through colostrum and milk to fight pathogens if illness is to arise. Although there has been extensive research into feeding LCP diets and supplementation of PRO in weaned pig diets to prevent PWD infection, little research has been done on the synergistic effects of LCP diets and PRO supplementation in the sow diet on gut health of their progeny.

The results of the current study show that diets with decreased protein content do not negatively impact the reproductive performance of the sow and LCP diets with PRO supplementation provided to the sow can reduce pathogenic bacteria in the gut of suckling pigs, leading to lower incidence of PWD infections. Of particular interest are the findings on the number of mummified piglets among treatment groups. In the present study, LCP-PRO diets reduced the number of mummified piglets compared to other treatment groups, which shows that reduced protein content does not negatively impact the reproductive performance of sows. In addition, piglets from sows supplemented PRO had an increased percentage of fecal DM. This suggests that the supplementation of PRO can improve digestion efficiency of both sows and piglets and improve the balance of the gut microbiome. The relative abundance of the diarrhea-causing pathogen *E. coli* was lower in piglets from sows receiving LCP diets compared to piglets from sows receiving HCP diets. High protein diets can cause higher proliferation of pathogenic bacteria because they cause a large abundance of non-digested substrates which can be used for pathogenic microbial fermentation allowing for increased growth. In conclusion, adapting the use of LCP sow diets supplemented with PRO has been shown to improve reproductive performance and suckling pig gut health, which can reduce the risk of PWD in nursery pigs.

# CHAPTER 5: FUTURE RESEARCH

Prior research has shown that low-protein diets and probiotic supplementation can improve the performance of weaned pigs. The current study has also shown that feeding LCP diets with PRO supplementation to sows can improve reproductive performance and improve growth performance and gut health of suckling pigs. Further research can be done to:

1. Repeat the current study with more than 2 levels of CP to determine the optimal level of CP to maintain sow performance and piglet performance and indices of gut health to reduce feed costs.
2. Repeat the current study with different treatments containing multiple strains of PRO in the sow diet to determine strains that have the most beneficial effects on sow and piglet performance and indices of gut health.
3. Investigate the effects of LCP diets with PRO supplementation in the sow diet on performance, indices of gut health and meat quality parameters in pigs from weaning to slaughter.

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