EVALUATION OF A NOVEL CORN FERMENTED PROTEIN INGREDIENT, PROBIOTICS, AND POSTBIOTICS ON NURSERY PIG GROWTH PERFORMANCE AND GUT HEALTH

BY

JUAN F. CASTILLO ZUNIGA

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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Ryan Samuel
Advisor Date

John Jaeger
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

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ABBREVIATIONS

AA	Amino acids
ADG	Average daily gain
ADP	Adenosine Diphosphate
ADFI	Average daily feed intake
ATP	Adenosine Triphosphate
BCAA	Branched-chained amino acids
BW	Body weight
BifPos	*Bifidobacteria*-based postbiotic
CFP	Corn fermented protein
CD	Crypt depth
DSAT	Differential sugar absorption test
DFM	Direct-fed Microbials
DDGS	Distillers dried grains with solubles
GIT	Gastrointestinal tract
G:F	Gain to feed ratio
IL-8	Interleukin 8
LAB	Lactic acid bacteria
LacPro	*Lactobacillus*-based probiotic
OTUs	Operational taxonomic unit
PWD	Postwean Diarrhea
SCFA	Short-chain fatty acids
SBM	Soybean meal
SPC	Soy protein concentrate
TJ	Tight junction
TLR2	Toll-like receptor 2
TNF-α	Tumor Necrosis Factor α
VH	Villus height
VCR	Villus height to crypt depth ratio

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ABSTRACT

EVALUATION OF A NOVEL CORN FERMENTED PROTEIN INGREDIENT, PROBIOTICS, AND POSTBIOTICS ON NURSERY PIG GROWTH PERFORMANCE AND GUT HEALTH

JUAN F. CASTILLO ZUNIGA

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Weaning is a necessary step in pig production. After weaning, pigs are subjected to various nutritional, psychological, and environmental stresses. The weaning period is an important period for the intestinal development of the pig. During this time, digestion, immunity, metabolism, and various other aspects of the pig change rapidly. Therefore, providing high-quality feed ingredients is crucial to encourage the newly weaned pig to start consuming solid feed. Two independent studies were conducted to determine if the inclusion of: 1) corn fermented protein (CFP) or 2) probiotics or postbiotics impact nursery pigs' growth performance and gut health. The first experiment utilized 1144 pigs distributed evenly into 44 pens (13 barrows and 13 gilts/pen; initial BW 6.0 ± 0.1 kg), with 286 pigs assigned per treatment and 11 replications. The four treatments were designed as a titration of CFP inclusion at 0%, 4%, 8%, and 12% in Phase 1 (3.62 kg/pig feed budget) and 0%, 2%, 4%, and 6% in Phase 2 (5.44 kg/pig feed budget) replacing soy protein concentrate. Pigs were fed a common diet through Phase 3 (10.28 kg/pig feed budget). A differential sugar absorption test (DSAT) was administered using a 5% lactulose and a 5% mannitol solution on the tenth day to pigs consuming the 0% and 12% CFP diets to determine gut integrity. Urine was collected to measure differences in sugar ratios

to assess gut permeability. Similar average daily gain (ADG) responses of pigs fed 4% and 8% CFP inclusion compared to the control diet were observed during Phase 1 ($P<0.01$). Pigs fed diets with 4% and 8% CFP inclusion in the second week of Phase 1 had a greater average daily feed intake (ADFI) than pigs fed 12% CFP diets ($P<0.01$) and had an intermediate ADFI compared to the control treatment pigs. At the end of Phase 2, pigs fed diets with 0%, 2%, and 4% CFP had greater ADFI than pigs fed diets with 6% CFP ($P<0.01$). After the first week of Phase 2, pigs fed 0%, 2%, and 4% inclusions of CFP had greater gain to feed ratio (G:F) than pigs fed 6% CFP ($P<0.01$). There was no difference between pigs fed the control diet and 12% inclusion when subjected to a DSAT test, suggesting no reduction in gut integrity when including CFP in the diet.

In the second experiment, 1,040 pigs were allocated to 40 pens of 26 pigs per pen, with a starting weight of 6.1 ± 0.1 kg. Pens were assigned to one of 4 dietary treatments fed over four dietary phases: 1) Control, 2) Control + 0.1% inclusion of Lactobacillus-based probiotic (0.1% LacPro), 3) Control + 0.2% inclusion of Lactobacillus-based probiotic (0.2% LacPro), or 4) Control + 0.2% inclusion of Bifidobacteria-based postbiotic (BifPos). Pens of pigs were weighed at barn entry, day 10, day 21, day 47, day 70, day 105, and day 135. Feed remaining on weigh day was calculated according to a prepared calibration curve accounting for the measured distance from the top of the feeder to the top of the feed and the density of the feed. Fecal samples were collected on day 10 and day 47 to evaluate microbial populations. To measure gut health parameters, on day 10, 40 pigs were euthanized, providing 10 jejunal and ileal tissue samples per treatment that were then measured for VH, CD, and VCR. On day 10, pigs provided the Control diet had lower ($p=0.05$) ADFI than pigs fed with diets containing the probiotics and postbiotic (150 vs 177 ± 3.38 g/d).

Histological analysis from day 10 indicated a greater ($P<0.02$) villus height to crypt depth ratio (VCR) in the ileal tissue from pigs fed 0.1% LacPro, 0.2% LacPro, and 0.2% BifPro compared to Control (1.04, 1.21, and 1.18 μm vs. 0.99 μm). An increased abundance ($P<0.03$) of Lactobacillaceae family in feces from 0.2% LacPro and 0.2% BifPos compared to Control (10.38% and 10.78% vs. 3.53%) on day 10 was observed. The increased gastrointestinal tract (GIT) surface area and greater abundance of Lactobacillaceae in both LacPro and BifPos-fed pigs may improve nutrient uptake and provide a more stable microbiota capable of degrading complex carbohydrates such as dietary fibers to help healthy pigs better adapt to diets during the weaning transition period.

In overall conclusion, supplementing nursery pig diets with CFP impacted ADG, ADFI, and G: F during the first two dietary phases, however it did not change BW throughout the trial. Feeding nursery pigs a *lactobacillus*-based probiotic and a *Bifidobacteria*-based postbiotic positively impacted ADFI in the first dietary nursery phase. Nevertheless, weight gain was unaffected. In both studies, gut health was measured. Corn-fermented protein did not compromise gut permeability, and LacPro and BifPos improved VCR in the ileum. Therefore, we can conclude that adding these feedstuffs can impact gut health, meaning that pigs may consume more feed early on and can potentially reduce weaning stress challenges.

LITERATURE REVIEW

1.1. Introduction

The weaning phase is one of the most eventful stages of swine production and can significantly impact a pig's life performance (Tang et al., 2022). Ensuring a smooth transition from the maternity ward to the wean to finish barn requires addressing factors such as diet changes, environmental changes, and new social interactions, which are all associated with weaning stress. We will discuss the stressors of the weaned pigs, explore feeding programs for nursery pigs, and examine the use of different feedstuffs and feed additives. Additionally, we will highlight the role of probiotics and postbiotics in mitigating weaning stress and its physiological impacts.

The nursery period is defined as the life cycle of a pig between six and eight weeks after birth. The pig is relocated to another facility, to reduce any pathogen load due to a safe distance between barns, and housed with piglets from other litters when they reach 21-23 day of age (Faccin et al., 2020). On average, the pig starts the nursery phase at 5.5 to 7 kg body weight and ends the nursery phase with an average weight between 23 and 28 kg (Pork Checkoff, 2021). The weaning period is also defined as the transition period when the newly weaned pig changes from a liquid milk-based to a solid plant-based diet (e.g. corn and soybean meal-based). The transition needs to be gradual, where the newly weaned pig has time to adjust to the change in diet (Menegat et al., 2019). One strategy in the transition period is to utilize a complex diet. Diet complexity refers to incorporating a variety of highly digestible feed ingredients that also includes milk-based ingredients into nursery pig diets. These complex diets are usually given to weanling pigs to ensure they receive high-quality feed and to boost their nutrient intake during the initial postweaning phase and to help with digestive transition from milk to cereal-based diets. However, because these diets

are costly, the level of complexity is often quickly reduced throughout the nursery period (Menegat et al., 2019). Throughout this literature review, we will also revise the importance of providing the newly weaned pig with a high-quality diet, its role in gastrointestinal tract (GIT) health, and how it can reduce the impact of weaning for the piglet.

1.2. Weaning Period Stressors

In commercial swine production, the weaning transition is the most critical period for a piglet's health and typically occurs between 3 and 4 weeks of age. During the weaning transition time, piglets are subjected to several stressors such as being separated from the sow, handling stress, different food sources, social hierarchy stress, co-mingling with pigs from different litters, different physical environment and housing, and, often, transportation to a new facility (Campbell et al. 2013). The weaning process in pigs triggers physiological shifts, including activation of the hypothalamic-pituitary-adrenal axis, changes in the sympathetic nervous system, and adjustments in growth-related hormone levels (Campbell et al., 2013). Moreover, weaning induces alterations in intestinal morphology, such as decreased villus height (VH), increased villus width, and deeper crypts which impact the enteric immune system. These changes can lead to diminished nutrient digestion and absorption, a propensity for post-weaning diarrhea, and heightened vulnerability to enteric infections (Pluske et al., 1997). Each stressor influences pig growth and health in the first weeks after weaning. This literature review will provide an overview of the impact of the stressors listed above on pig growth and/or health with an emphasis on digestive development and how the diet can be used to help pigs adapt and ensure good growth and health during the transition period.

1.2.1. Sow Separation, Environmental, Transportation, Handling, and Social Stress

Separation of the piglet from its mother can cause sudden distress evidenced by prolonged vocalization, restless activity, and long-term behavioral effects (Weary et al., 1999). In addition, the suckling pig has, up to this point, received nutritional support almost solely from the sow's milk (Weary et al., 1999).

Although newborn piglets initially struggle with regulating their body temperature, their thermoregulatory capacity significantly improves by the time of weaning. At this stage, they have developed a layer of fat for insulation and acquired an energy intake level that supports metabolic functions and growth. This enables them to generate sufficient heat for thermoregulation. However, alterations in diet, such as that associated with weaning, can markedly impact heat production, influenced by changes in metabolic energy intake (Le Dividich and Herpin, 1994). Studies have recommended the lower critical temperature is between 26 and 28 °C for the first week following weaning (Le Dividich & Herpin, 1994). In the next 2 weeks, the lower critical temperature is near 24 °C, and then each subsequent week, a 2 to 3 °C reduction can occur until typical finishing conditions are achieved (Le Dividich & Herpin, 1994).

Transportation and handling of weaned pigs is also a major stressor for the weaned pig. Pigs are predominantly transported to separate production facilities at weaning to reduce vertical disease transfer and improve pigs' early post-weaning growth and productivity potential (Sutherland et al., 2014). During transportation, pigs may encounter various stressors, such as handling during loading and unloading, temperature changes, interaction with unfamiliar pigs causing social stress, deprivation of feed and water, exposure to a new environment, and vibrations and

noise. These factors can potentially diminish welfare and raise the risk of illness and death among the pigs via a reduction in feed intake and more susceptibility to pathogens (Sutherland et al., 2014).

Upon arrival at a new facility, weaned pigs are mixed into pens with other weaned pigs. This can be categorized as a stressor because pigs establish a dominance hierarchy among the group and compete for resources, such as food, territory, and mates (Tong et al., 2020). This aggression among pen mates occurs most during the first two weeks post-wean (Stookey & Gonyou, 1994). Upon moving and mixing unfamiliar pigs, there is antagonistic behavior shown by increased saliva cortisol concentrations (Merlot et al., 2004), as well as inhibited growth and ear and body lesions (Escribano et al., 2019).

1.2.2. Transition from a Milk-based Diet to a Cereal-based Diet

At weaning, digestive enzymes adapt from processing milk to breaking down starch and plant proteins (Campbell et al., 2013). Pigs, before weaning, rely on the sow's colostrum for humoral immune protection through the intake of immunoglobulin G until the immune system is mature enough to produce antibodies to fight off foreign antigens (Rooke & Bland, 2002). In addition, they face a new environment where they shift from frequent meals with the sow to fewer meals amidst increased competition (Campbell et al., 2013). They also need to learn to eat from feeders and drink from new water sources. This can lead to stress-related issues, translating to reduced feed intake and immune system dysfunctions reducing the pig's health (Campbell et al., 2013).

1.2.3. Importance of Feed Intake During the First Weeks Post-Wean

The abrupt change to a solid dry diet at weaning that is less digestible and palatable than the sow's highly digestible and palatable milk diet before weaning has implications on weaned pig feed intake during the transition period. Le Dividich and Sève (2000) highlighted that metabolizable energy (ME) intake decreases by 30–40% compared to pre-weaning energy intake, taking approximately 2 weeks post-weaning to fully recover to pre-weaning ME intake levels. This reduced feed intake during the post-weaning period may contribute to intestinal inflammation, adversely affecting intestinal integrity by reducing villus height and increasing crypt depth (Upadhaya & Kim, 2021).

The impact of low feed intake during weaning is also evident in reduced growth performance. A weight loss of 100–250 g body weight (BW) can be expected on the first day of weaning, regardless of wean age, but its recovery within 4 day is common (Campbell et al., 2013).

On average, about 10% of pigs do not ingest feed during the first 48 hours after weaning; this can disrupt the small intestine, making them more susceptible to infections and increased pathogen entry (Wijtten et al., 2011). Adequate feed intake levels prevent the loss of intestinal barrier function due to the preservation of intestinal structure, this limits the passage of pathogens into the weaned pigs' system (Wijtten et al., 2011).

1.3. Functions of the Gastrointestinal Tract and its Physiological Changes at Weaning

The GIT serves several vital functions, such as food ingestion and digestion, nutrient absorption, secretion of water and enzymes, and waste product excretion (Ogobuiro et al., 2023). The GIT is responsible for nutrient absorption; most nutrients are absorbed in the second and third part of the jejunum, and the rest are absorbed in the ileum. In pigs, the GIT is the largest immune organ, housing over 70% of the body's immune cells (Mason et al., 2008). Additionally, the GIT plays a crucial role in maintaining immune homeostasis (Szabó et al., 2023). The GIT is divided into four layers: the mucosa (epithelium, lamina propria, and muscular mucosae), the submucosa, the muscularis propria (inner circular muscle layer, intermuscular space, and outer longitudinal muscle layer), and the serosa (Rao et al., 2010).

The intestinal epithelium is a single-cell layer of the GIT serving as both a barrier against the external environment and allowing the absorption of nutrients and water (Szabó et al., 2023). Between the cells of the GIT, tight junctions (TJs), a protein complex between two cells that create a seal to prevent any leakage through the cell membranes exist (Anderson & Van Itallie, 2009).

The TJ proteins are crucial in controlling the passive diffusion of ions, small solutes, and pathogenic organisms through the paracellular pathway, thereby maintaining proper epithelial function. These proteins, which form complex structures of transmembrane and membrane-associated proteins, act as ion channels and barriers against harmful molecules. When intestinal permeability is increased, it can lead to inflammatory responses by allowing the entry of toxins, allergens, viruses or bacteria. Factors such as age, diet, pathogens, and diseases can compromise the function of these TJs and, consequently, the intestinal barrier (Wang et al., 2019).

In piglets, the immature and underdeveloped intestine is susceptible to damage from various stresses, infections, and dietary factors. Probiotics, amino acids, fibers, oligosaccharides, and specific micronutrients have been shown to play a role in regulating intestinal TJs via enhanced barrier integrity and increased expression and distribution of TJ proteins (Wang et al., 2019).

Key indicators of intestinal health and absorption status include factors related to intestinal morphology, such as VH, crypt depth (CD), and the villus height to crypt depth ratio (VCR) (Campbell et al., 2013). A decrease in VH and VCR indicates compromised intestinal mucosal function, reducing intestinal digestion and absorption capacity. Conversely, higher VH, VCR, and lower CD signify improved intestinal function (Campbell et al., 2013). Weaned animals commonly experience alterations in the villus-crypt structure, including intestinal villus shedding, crypt hyperplasia, and intestinal mucosa atrophy. These changes can undermine the intestinal mucosal barrier function and digestive and absorptive capacity (Campbell et al., 2013). For example, Bomba et al. (2014) revealed a significant decrease in VH and VCR in the ileum of piglets five day after weaning compared to before weaning. Similarly, Hu et al. (2013) confirmed the deterioration of intestinal morphology induced by weaning, demonstrating a decrease in VH and VCR on day 3 and 7 postweaning compared to the preweaning stage. The VH and CD did not return to preweaning levels until day 14 after weaning. Additionally, Boudry et al. (2004) reported structural changes induced by weaning, with the VH of the jejunum still significantly lower on day 15 after weaning compared to preweaning. Weaning stress has been observed to reduce the relative weight of the small intestine as well, with the total weight of the intestine 15 day after weaning being only 50% of that before weaning (Tang et al., 2022).

1.3.1. Effect of Weaning on Digestive Enzyme Activity and pH

There is a negative effect during the transition period, where the lack of solid feed intake after weaning affects digestive enzyme secretion of the GIT. One week after weaning, the activities of digestive enzymes decrease to one-third of those prior to weaning, which leads to poor nutrient digestion and higher rates of diarrhea (Shi et al., 2022). Gastrointestinal tract digestive enzymes play a key role in regulating animal growth and development by enhancing feed efficiency through digestion and subsequently modulating nutrient metabolism (Liu et al., 2021). Absorptive intestinal epithelial cells dominate the crypt-villus axis and secrete various digestive enzymes, including disaccharidases, peptidases, and phosphatases. During the first three weeks after birth, piglets' digestive systems develop rapidly due to sufficient nutrition from sows. This leads to significantly increased activities of enzymes like intestinal lactase, protease, and lipase (Liu et al., 2021). However, after weaning, the activities of these enzymes, particularly those on the brush border of the intestinal mucosa, undergo dramatic changes due to dietary changes. This alteration in enzyme activity, particularly the decline in disaccharidases, is linked to post-weaning diarrhea in piglets (Shang et al., 2020). Alkaline phosphatase, a key enzyme in the small intestinal villus epithelium, enhances nutrient uptake and conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). Weaning stress negatively impacts intestinal digestion and absorption functions. The reduction in digestive enzyme activity after weaning is likely due to weaning stress adversely affecting intestinal morphology, inhibiting endogenous enzyme secretion (Liu et al., 2021).

Following digestive transition to plant-based diets, the primary digestive enzymes are amylase, lipase, and protease. Amylase breaks down starches and carbohydrates

into sugars, lipase breaks down fats and oils into glycerol and fatty acids, and protease breaks down proteins into amino acids. The most significant protein-digesting enzymes in the pig's intestine are trypsin and chymotrypsin (Szabó et al., 2023).

Nutrient digestion is intricately linked to the function of digestive enzymes. Various factors such as pH, dietary composition, salinity, developmental stage, and diet composition can influence the activity of digestive enzymes in the gut (Shi et al., 2022). Changes in the physicochemical environment of the gut can consequently impact enzyme activity. Particularly, maintaining an acidic pH in a piglet's gut (1.15-4) is crucial for optimal production and function of digestive enzymes, as many enzymes exhibit enhanced activity under acidic conditions (Shi et al., 2022). During the initial 0-4 weeks of life, the activities of lipase and trypsin in a piglet's gut increase twofold each week (Shi et al., 2022). Consequently, during the first-week post-weaning, piglets exhibit low utilization rates of carbohydrates and fats in non-dairy feeds, struggling to digest plant proteins or adapt to solid feeds, leading to indigestion and diarrhea. During this phase, gastric acid is relatively mild, with limited lactic acid production in the intestines (Shi et al., 2022). Additionally, some gastric acid combines with feed components, resulting in a higher intestinal pH than the preweaning stage. Research indicates that lactic acid, derived from lactose fermentation, plays a crucial role in maintaining intestinal acidity, which supports the production and function of digestive enzymes (Shi et al., 2022). Elevated pH levels impede digestive enzyme activity and upset the equilibrium of GIT microflora, leading to an imbalanced environment. Consequently, this disruption reduces the effective area for digestion and absorption, damages the intestinal mucosal barrier, and weakens immune responses (Shi et al., 2022).

1.3.2. The Gastrointestinal Tract and its Microbiome

Like humans, pigs possess a complex and diverse community of microorganisms in their GIT, crucial for immunity, physiological processes, and nutrient metabolism. The GIT microbial community's diversity, composition, and function are influenced by diet, age, stress, and the environment. These factors can directly or indirectly impact the host's metabolism, immune response, and intestinal homeostasis, creating a cross-talk between the GIT microbiota and the host (Luo et al., 2022). The GIT has many microorganisms that contribute to intestinal mucosal immunity. The GIT microbiome and the GIT immune system complement each other, where the immune system contributes to regulation of the distribution and composition of the microbiota by secreting various immune effector factors, which include cytokines, chemokines, antibodies, and signaling molecules. The GIT microorganisms simultaneously promote the differentiation of immune cells, such as regulatory T cells (Szabó et al., 2023).

The transition from a milk-based to a solid diet at weaning leads to significant changes in the GIT microbiota. During the first week after weaning, the dramatic shift in the GIT microbiota can negatively affect growth and health (Luo et al., 2022). Healthy piglets develop a GIT microbiota rich in bacteria like *Prevotella*, *Roseburia*, and *Lachnospiraceae*. These families of bacteria help digest complex carbohydrates and produce short-chain fatty acids which promote intestinal health and energy metabolism. Conversely, piglets experiencing post-weaning diarrhea (PWD) often exhibit increased levels of harmful bacteria like *Campylobacter* and decreased beneficial bacteria (Luo et al., 2022).

A well-balanced diet is one of the most effective ways to counteract the many stressors that the weaned pigs face when entering this new production stage (Luo et al., 2022). Weaned pig diets most often have highly digestible and palatable ingredients to stimulate feed intake, which may reduce further disease and/or GIT dysfunctions and reduce the abrupt changes that the pig is going through (Luo et al., 2022). Therefore, a well-planned feeding program must be implemented to meet the weaned pig's nutritional requirements.

1.4. Feeding Program for Nursery Pigs

A balanced swine diet provides the necessary nutrients in the appropriate proportions based on the available information (e.g. NRC, 2012) to adequately meet the animal's nutritional requirements (LaRosae, 2022). The primary goals of a nursery feeding program are to maximize feed intake and help pigs maintain a healthy GIT (DeRouchey et al., 2010). As noted previously, pigs at this stage face numerous challenges, such as limited ability to utilize dietary fat and low activity of digestive enzymes such as amylase, maltase, and sucrase (Hunting et al., 2021).

Feeding the nursery pig also requires dietary phase adjustments throughout this period. This is necessary to base diets on feed budgets and or weight ranges to closely meet the pig's nutrient requirement, to optimize economic output. Typically, the nursery phase feeding program consists of using a budget depending on the weight of the piglet; as the pigs become heavier at weaning, the amount of the initial nursery diets are reduced, as well as the complexity of the ingredients (Menegat et al., 2019). The weaned pig, on average, arrives at 5 to 6 kg that is where the first nursery phase begins; this phase is particularly important because it is important to provide high-quality ingredients to stimulate feed intake (Menegat et al., 2019). The Phase 1 diet

for piglets, from weaning to reaching 6 kilograms requires a concentration of amino acids (eg.1.50% SID lysine at 6 kg, 1.35% SID lysine at 9 kg, and 1.23% SID lysine at 18 kg of BW), As reported by the NRC (2012). It combines various protein sources to meet nutritional needs and stimulate feed intake (DeRouchey et al., 2010).

Nursery diets are typically called a complex diet due to the nursery pig's elevated nutrient requirements, with highly digestible specialty feed ingredients with the objective of improving feed intake right after weaning (NRC, 2012). Higher diet complexity refers to a greater variety of ingredients where feedstuffs are included for their high nutrient availability and reduction of anti-nutritional factors (Rodrigues et al., 2022). Animal-based ingredients provide high-quality protein sources without antinutritional compounds and have been shown to enhance health and growth performance in nursery pigs (Rodrigues et al., 2022). Dairy products, such as lactose and whey, improve palatability and facilitate the transition from milk-based diets post-weaning. Pigs fed complex diets tend to gain more weight and consume more feed post-weaning compared to simpler diets. Pigs may also exhibit higher nutrient digestibility and energy intake, with a more balanced protein metabolism (Rodrigues et al., 2022). Additionally, newly weaned pigs have infrequent visits to feeders. On average, they visit feeders only once daily. Therefore, the diet is more nutrient dense so the piglet can fulfill its nutrient requirements in small bites of feed (van Kempen et al., 2023).

Another important aspect of feeding nursery pigs is delivering their feed in crumble form for the first two nursery phases. Crumbles are made from whole pellets that are broken down into smaller sizes. The advantage of the pellet is that it improves feed efficiency, reduces feed wastage, and improves the diet's flowability characteristics (DeRouchey et al., 2007).

1.4.1. Primary Feedstuff Composition of Nursery Diets

Common protein sources used in weaned pig diets include spray-dried animal plasma, fish meal, dried whey, blood cells, poultry meal, whey protein concentrate, spray-dried blood meal, soybean meal, and processed soy products (DeRouchey et al., 2010). On a relative scale, the price of these high-quality protein sources is typically 25% to 30% more than soybean meal (Ycharts, 2024).

Spray-dried animal plasma effectively boosts feed intake and improves intestinal health and immune function. Studies have shown that animal plasma can increase feed intake due to improved palatability of the diets (Ermer et al., 1994). Animal plasma can also increase the number of *Lactobacilli* in the ileal and cecal content in piglets (Torrallardona et al., 2003). Spray-dried blood meal and blood cells are rich in lysine but limited in their methionine and isoleucine content thus diet inclusion may be limited or necessitate supplemental methionine to meet target diet nutrient levels (DeRouchey et al., 2010). High-quality fish meal, poultry meal, or whey protein concentrate are also included for their digestibility and ability to meet amino acid requirements (DeRouchey et al., 2010).

Lactose is crucial to promote pigs' growth after weaning because it is a palatable and easily digestible energy source that eases the transition from milk to solid feed (Zhao et al., 2021). Lactose, a disaccharide found in milk, serves as the primary carbohydrate source for infant mammals. In the small intestine, lactase breaks down lactose into glucose and galactose, providing easily absorbable energy for young mammals (Zhao et al., 2021). Some of the dietary lactose is fermented by bacteria such as *Lactobacillus* in the stomach, producing lactic acid and small amounts of acetate (Zhao et al., 2021). The activity of endogenous lactase in pigs significantly

decreases after weaning thus lactose is typically included in the first few diet phases after weaning (Zhao et al., 2021). The Phase 1 diet typically contains 20-25% lactose, primarily sourced from dried whey (Zhao et al., 2021). Common lactose sources are crystalline lactose, whey permeate, and dried whey (Menegat et al., 2019).

For carbohydrates to serve as an immediately available energy source for weanling pigs, they must be highly digestible, especially to counter the low feed intake following weaning. In addition to lactose, weanling pig diets can include sucrose and dextrose. Sucrose, a simple carbohydrate derived from sugar cane or sugar beet, offers an easily digestible energy source from glucose and fructose. Incorporating 5 to 10% sucrose in initial nursery diets has been shown to enhance the growth performance of weanling pigs as effectively as lactose (Coffey et al., 2000). Dextrose, a glucose usually derived from corn, offers pigs a readily digestible energy source. Like sucrose, dextrose, dextrose-based products, and other simple sugars, they can partially substitute lactose in early nursery diets without negatively affecting growth performance (Coffey et al., 2000).

Weanling pigs in the early post-weaning period require a more digestible fat source rich in unsaturated and short-chain fatty acids for efficient energy utilization. Fat addition is mainly used to improve the pelleting process of initial nursery diets with high levels of lactose (Lauridsen et al., 2007). Among the commonly used fat sources in pig feed are animal fat (lard, tallow), vegetable fat sources (coconut oil, palm oil, palm oil mix, corn oil, rapeseed oil, and soybean oil), and marine fat sources (fish oil) (Lauridsen et al., 2007). In general, vegetable fat sources (in particular, corn oil, coconut oil, and soybean oil) are more digestible than animal fat sources (Lauridsen et al., 2007).

1.4.2. Soybean Meal Inclusion in Nursery Diets

Soybean meal (SBM) is a major co-product of soybean oil extraction and is the most common plant protein ingredient used in swine diets because its amino acid profile is similar to animal-derived protein ingredients, and its market supply is relatively stable (Banaszkiewicz, 2011). However, inclusion in the first few phases of the weaned pig feeding program is limited due to the antinutritional factors this protein source presents. The goal of including SBM in early nursery diets is to gradually expose the piglet to increasing levels of SBM to create a tolerance effect (Menegat et al., 2019).

The concern with SBM in weaned pig diets is the presence of antigenic proteins glycinin and β-conglycinin, which trigger a hypersensitive immune response in the gastrointestinal tract of weaned pigs (Li et al., 1991). This hypersensitivity leads to abnormal morphology in the small intestine and a reduction in absorptive capacity (Goebel and Stein, 2011). For example, Faccin et al. (2023) reported that nursery pigs fed high levels of SBM (36.2%) had decreased ADG, ADFI, and final body weight. Lawrence et al. (2004) also found negative impacts of SBM inclusion on growth performance, specifically G:F, when including 40% SBM during the first 14 day post-weaning. Common inclusion levels of SBM across the weaned pig feeding program are 12-15% for 5 to 8 kg pigs, up to 20% for 8 to 12 kg pigs, and 26 to 28% for 12 to 23 kg pigs. (DeRouchey et al., 2010)

Some swine producers turn to alternative protein sources to decrease SBM levels in the weaned pig diet. However, using products such as enzymatically treated SBM comes with an increased diet cost, and its impact on the growth performance of weanling pigs is inconsistent (Zhou et al., 2011; Ma et al., 2019a; Ruckman et al.,

2020). Therefore, exploring alternatives to enzymatically treated SBM and further processed soybean products may prove advantageous for swine producers.

1.4.2.1. Soy Protein Concentrate

Soy protein concentrate (SPC) is produced from dehulled and defatted SBM, removing the water/alcohol soluble non-protein components, as well as the soluble carbohydrates (NRC, 2012). SPC contains a minimum of 65% CP (NRC, 2012). The process of converting SBM to SPC also removes some bitter off-flavors, potentially improving feed intake. Studies have shown that pigs fed diets with SPC exhibit higher weight gain and feed efficiency compared to those with SBM (Zixiao et al., 2023).

In the production of SPC and isolate, allergenic proteins, and indigestible carbohydrates from soybeans are largely eliminated (Stein et al., 2016). Incorporating approximately 14% of SPC into nursery diets has enhanced growth performance compared to SBM (Lenehan et al., 2007). However, being cautious with higher inclusion rates of SPC is important, as it may impact palatability and reduce feed intake (Lenehan et al., 2007).

1.4.3. Corn Co-Products in Swine Feed

Corn co-products have been used in swine feed for over fifty years, but the rise of the fuel ethanol industry in recent decades has significantly increased the availability of these co-products for livestock and poultry industries (Stein, 2011). Distillers dried grains with solubles (DDGS) is the primary by-product of ethanol production, and its US production now surpasses that of (Stein, 2011). DDGS are a by-product of corn used in the dry grinding process for ethanol production. In 2021, the United States produced over 15 billion gallons of ethanol from corn, resulting in the generation of

44 million metric tons of DDGS (U.S. Grains Council, 2022). Distiller's dried grains with solubles are an excellent feed ingredient; it can be included in diets for weanling pigs at levels up to 20% to 30% without negatively impacting growth performance (NRC, 2012).

Corn-fermented protein (CFP) is an innovative protein ingredient produced by reintroducing various protein and yeast fractions from ethanol production back into HPDDGs, resulting in a product that boasts up to 50% CP and 2% lysine. This enhancement significantly increases the nutritional value of CFP, making it a valuable ingredient in animal feed formulations (Stas et al., 2022).

1.4.3.1. Corn Fermented Protein

As a high-quality protein source, CFP has the potential to serve as an alternative protein source to feed nursery pigs, it is a good source of lysine and methionine (containing approximately 20% to 25% of yeast content). When considered on a dry matter basis, its composition includes 53% protein, 6% fat, and 3% ash (Kilburn-Kappeler et al., 2022).

However, corn-derived co-products pose a potential challenge due to their high levels of leucine, which can lead to an imbalance in branched-chain amino acids (BCAA) in relation to lysine (Cemin et al., 2019). The surplus dietary leucine found in corn-based co-products may disrupt the utilization of other BCAA, such as valine and isoleucine, ultimately elevating their dietary requirement (Cemin et al., 2019). This is due to the effect of high levels of leucine inhibiting the absorption of valine and isoleucine, creating an antagonistic effect. If there is an excess of leucine, there will be an increased stimulation of enzymatic activity, increasing the catabolism of the other two BCAA (Cemin et al., 2019).

Increasing levels of other BCAA in diets with excess leucine can enhance the growth performance of finishing pigs (Kerkaert et al., 2021). However, limited data are available to ascertain whether a similar approach to increasing other BCAA would improve the performance of nursery pigs consuming diets with elevated Leu levels (Stas et al., 2021).

Nursery pigs require a balanced intake of amino acids for optimal growth and health (Stas et al., 2021). Notably, the amino acid ratios in CFP, relative to lysine content, are greater than those found in conventional DDGS sources. Moreover, CFP stands out for its elevated concentrations of Leu, compared to SBM (5.84% vs. 3.35%), which have significant implications for protein synthesis, particularly in the intestinal tract and liver (Stein, 2022).

The regulation of feed intake, important for weaned pigs, is influenced by serotonin levels. Serotonin is a neurotransmitter that affects appetite and satiety. Higher serotonin levels are associated with reduced appetite and decreased food intake (Stein, 2022). Tryptophan acts as a regulator of feed intake by enhancing serotonin signaling in the brain. Excess dietary Leu may also reduce serotonin synthesis in the brain (Wessels et al., 2016).

Corn-fermented protein has an elevated total dietary fiber content, ranging between 30-35% compared to SPC which contains 23%. While the total protein concentrations in CFP are comparable to those in SBM, variations exist in Lys and Trp concentrations. These differences emphasize the need for careful consideration of the nutrient profile of corn protein when formulating diets for weanling pigs (Stein, 2022).

1.5. Feed Additives in Swine Diets

Feed additives are effective in regulating intestinal environments, enhancing the pigs' immune system, and reducing the negative impacts of weaning and other related environmental challenges (Liu et al., 2018). The Official Journal of the European Union (2003) defines feed additives as substances, microorganisms, or preparations, other than feed material and premixtures, added to feed or water to perform various functions. These functions include improving feed characteristics, enhancing animal product quality, satisfying animal nutritional needs, benefiting the environment, boosting animal performance or welfare by affecting gut flora or digestibility and providing coccidiostatic effects. Importantly, feed additives should not harm animal or human health or the environment, mislead users, or negatively impact the distinctive features of animal products.

Numerous non-antibiotic feed additives have been suggested or examined to enhance growth performance, regulate gut microbiota, boost immunity, address environmental challenges, and improve reproductive performance (Richert et al., 2006). Not all feed additives are the same or provide a beneficial response, and therefore, choosing a suitable product will depend on the farm's specific situation and needs (Jay et al., 2009).

In-feed antibiotics are added to pig feed primarily to prevent colonization by pathogenic microorganisms in the intestine and to aid in controlling respiratory and reproductive bacterial diseases (Liu et al., 2018). Some suggested mechanisms through which antibiotics can foster growth performance of pigs involve preventing subtle infections caused by bacteria, minimizing the production of metabolic by-products that could hinder pig growth, hindering microbial growth to boost nutrient

availability for pigs, and enhancing the absorption and utilization of nutrients through the intestinal wall (Cromwell, 2002). The use of antibiotics in animals has raised concerns that the selective pressure on the bacteria population promotes antibiotic resistance (Lekagul et al., 2019). As of June 2023, the U.S. Food and Drug Administration declared that all medically important antibiotics in livestock production would be required to have a veterinary prescription and no longer be sold as over-the-counter products (Center for Veterinary Medicine, 2021).

Acidifiers can be both organic and inorganic acids. Examples of organic acids are formic, fumaric, lactic, benzoic, propionic, and citric acids, while inorganic acids include hydrochloric, sulfuric, and phosphoric acids. Salts derived from acids, such as calcium-formate, potassium-formate, sodium-formate, and sodium-fumarate, are also utilized as acidifiers (Richert et al., 2006). Commercially available blends of acidifiers are common. Additionally, certain commercial acidifiers incorporate protected acids coated with fatty acids or other molecules to facilitate targeted release in the gut and enhance effectiveness (Upadhaya et al., 2014). Acidifiers are mostly used in nursery diets to improve growth performance and promote the growth of beneficial bacteria while inhibiting pathogenic bacteria (Upadhaya et al., 2014).

Enzymes are active proteins that break down feed components, facilitating the release of nutrients for digestion and absorption (Thacker, 2013). In swine diets, supplementing exogenous enzymes in pig diets is used to increase dietary energy and fiber digestibility (Aranda-Aguirre et al., 2021). Exogenous enzymes break down feed components resistant to the body's natural enzymes, neutralize antinutritional factors, and supplement endogenous enzymes in inadequate quantities (Thacker, 2013). Exogenous enzymes that can be introduced into pig diets include phytases, carbohydrases, proteases, and lipases (Munezero, 2022). Phytase is an enzyme widely

used for its effectiveness in releasing phosphorus from phytate (Thacker, 2013). It is one of the most commonly utilized enzymes, accounting for 60% of the sales market (Adeola & Cowieson, 2011). Phytase acts to hydrolyze phytate to release phosphate, improving the digestibility of P, calcium, and AA (De Faria et al., 2015).

Direct-fed microbials (DFM), also known as probiotics, are often combined with yeasts and prebiotics (non-digestible food ingredients that stimulate the growth and activity of bacteria). Probiotics are live microorganisms that when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). They increase beneficial gut bacteria, primarily enhancing short-chain fatty acid (SCFA) production to reduce pH, inhibit enteric pathogens, stimulate intestinal cell proliferation, and maintain gut integrity (Amachawadi et al., 2018).

Probiotics are being used as feed supplements because it has been demonstrated that they provide several health benefits, including preventing diarrhea, modulation of GIT microbiota, and potentially acting against various infectious agents (Hill et al., 2014). The following text will review the use of probiotics and postbiotics in weaned pigs and their functions contributing to the wean pigs' health.

1.6. Probiotics and Postbiotics

Probiotics are defined by the FAO/WHO as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2001). These live microorganisms improve the intestinal microbial flora (Kenny et al., 2011; Bajagai et al., 2016). Several commercial probiotic products may contain bacterial cultures, yeast cells, or both that stimulate microorganisms capable of modifying the gastrointestinal tract environment to improve the host's health status

and feed efficiency. Probiotics may also include enzymes and crude extracts in addition to the live microorganisms (Bajagai et al., 2016).

Probiotic bacteria utilized in swine farming must possess specific characteristics categorized into four main attributes (Gaggía et al., 2010). Firstly, they should demonstrate the ability to colonize or be metabolically active in the gut, requiring resistance to gastric acid and digestion to interact with the host gut effectively. Secondly, they should contribute to health by directly stimulating the host immune response or indirectly reducing the burden of pathogenic bacteria. Thirdly, industrial applicability is crucial, including factors such as scalable production, long shelf-life stability (viable for 4 months), suitability for farm conditions, and favorable organoleptic properties for animal consumption. Finally, safety is paramount, addressing the animal's health in terms of being non-toxic and non-pathogenic and considering public health concerns such as the absence of transmissible antibiotic-resistance genes.

Probiotics are mainly composed of lactic acid bacteria (LAB), a group of gram-positive, acid-tolerant, generally non-sporulating, non-respiring rod-shaped (bacillus) or spherical (coccus) bacteria that are associated with their common metabolic and physiological characteristics. Through carbohydrate fermentation, LAB produces lactic acid as the main metabolic end-product (Yang et al., 2015). Lactic acid bacteria include various major genera, including *Lactobacillus* spp, *Bifidobacterium* spp, *Lactococcus* spp, *Lactosphaera* spp, *Leuconostoc* spp, *Melissococcus* spp, *Oenococcus* spp, *Pediococcus* spp, *Streptococcus* spp, and *Enterococcus* spp (Yang et al., 2015).

The application of probiotics in pig farming is geared towards promoting a balanced gut microbiota, ultimately enhancing the overall health and well-being of the animals (Cho et al., 2011).

Postbiotics, the bioactive compounds produced by probiotic microorganisms during fermentation, are gaining attention for their potential health benefits. Several postbiotic terms have been used, including 'Tyndallized probiotics,' 'Heat-killed probiotics,' 'Paraprobiotics,' and 'Bacterial lysates.' Despite increasing research and publications on postbiotics, their precise definition remains debated. Tsilingiri et al. (2013) first coined the term "postbiotics," referring to metabolic products derived from probiotics that benefit the host. In 2019, the International Scientific Association of Probiotics and Prebiotics (ISAPP) defined postbiotics as "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host." Probiotics' safety is crucial for their use, but concerns such as genetic stability, infectivity, and toxin production exist (Zhong et al., 2022). Non-replicating and non-producing microorganisms or their products, postbiotics avoid these issues, though they may release toxic metabolites needing further assessment. Probiotics' viability can decrease by the end of their shelf life, leading to excess dosing to ensure effectiveness. This issue is less relevant for postbiotics, which remain stable and effective throughout their shelf life, potentially making them more advantageous for precise dosing and application (Zhong et al., 2022).

At weaning, the sudden shift from a milk diet, which is a liquid containing easily digestible nutrients, to solid feed primarily made up of plant-based ingredients with polysaccharides, is likely the most disruptive event for the developing microbiome (St. Pierre et al., 2023). During the post-weaning period, gut microbial compositions become more diverse compared to earlier stages, complicating the identification of

core bacterial groups. This increased variation is partly due to various dietary strategies used to help weaned pigs adapt to new diets. Specialty ingredients like fishmeal, whey, and oats significantly influence which bacterial species thrive (St. Pierre et al., 2023). In response to the dietary shift, bacterial groups capable of metabolizing plant polysaccharides, such as *Prevotellaceae*, increase in abundance (St. Pierre et al., 2023). Additionally, microbial groups that metabolize smaller feed compounds or end products from other microbes, such as *Veillonellaceae* and *Oscillospiraceae*, increase (St. Pierre et al., 2023). Other predominant groups include *Ruminococcaceae*, *Lachnospiraceae*, and *Lactobacillaceae*, the latter thriving due to delayed expression of host alpha-amylase, allowing dietary starch availability (St. Pierre et al., 2023). The early post-weaning period often sees increased diarrhea, linked to higher dysbiosis risk from diet transition and stress, with higher abundances of *Sutterella*, *Campylobacter*, and *Fusobacteriaceae* species (St. Pierre et al., 2023).

1.6.1. Benefits of the Use of Probiotics and Postbiotics in Nursery Diets

Probiotics can modulate the GIT microbiota, leading to heightened intestinal immunity, enhanced disease resistance, decreased pathogen shedding, reduced disease symptoms, and improved health (Liao et al, 2017). Management-wise, adding this feed additive to swine diets has significantly enhanced the ADFI and feed conversion ratio (Liao et al., 2017). The gut microbiota supports the host by producing vitamins, utilizing indigestible feed ingredients, detoxifying feed components, forming a protective microbiota layer, generating natural antibiotics, maintaining gut barrier function, and promoting an anti-inflammatory response (Fouhse et al., 2016).

The established gut microbiota forms a complex micro-ecosystem coexisting with the pig host (Guevarra et al., 2019). Symbiosis, or balanced coexistence, is

crucial for normal gut functioning. Animals raised without bacteria experience delays in developing adult gut morphology, digestive physiology, and normal immune function (Guevarra et al., 2019).

In natural settings, harmful microorganisms can invade the GIT, leading to dysbiosis. This colonization results in issues like gas bloating, diarrhea, constipation, ulcers, and more severe outcomes. Dysbiosis compromises the pig's ability to utilize dietary nutrients, impacting growth efficiently. Controlling the GIT micro-ecosystem in modern pig production systems aims to prevent diarrhea, enhance health, and improve growth performance (Liao et al., 2017).

In addition to their growth-promoting properties, there is documented evidence that probiotics may also increase nutrient digestibility (Yirga, 2015). Previous studies have demonstrated that supplementing the diet with complex probiotics can enhance dry matter apparent total tract digestibility in weaned pigs (Ahmed et al., 2014; Choi et al., 2016). Additionally, other studies have reported that dietary supplementation with complex probiotics stimulates the total tract digestibility of nitrogen or gross energy in weaned pigs (Zhao & Kim, 2015).

Several postbiotic components interact with the host, giving them numerous health benefits. One of the components is exopolysaccharides. Exopolysaccharides are high molecular weight carbohydrate polymers produced and secreted by microorganisms, particularly lactic acid bacteria (LAB) such as *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* (Nguyen et al., 2020). These polymers possess remarkable capabilities, including water-binding, retention, swelling, and gelation, which are crucial for biofilm formation on bacterial surfaces. The health benefits are extensive, encompassing antimicrobial, immunomodulatory, and anti-inflammatory

effects (Zhong et al., 2022). For instance, exopolysaccharides derived from *L. rhamnosus* exhibit antibacterial activity against pathogens like *Salmonella* and *E. coli*, prevent bacterial adhesion, and support epithelial barrier integrity in the gut (Gao et al., 2017).

Another postbiotic component is cellular wall fragments. Bacteria like *Lactobacillus* and *Bifidobacterium* have cell walls that contain peptidoglycan, teichoic acids, and proteins (Chapot-Chartier & Kulakauskas, 2014). Peptidoglycan constitutes a significant portion of the cell wall in LAB. It plays a vital role in maintaining immune balance and inhibiting inflammatory cytokines through the toll-like receptor 2 (TLR2) pathway, making peptidoglycan an essential component in modulating the immune response (Shida et al., 2009). Teichoic acids are crucial for cell adhesion, inflammation, and immune activation; they can induce cytokine release via the TLR2 pathway (Shida et al., 2009).

Postbiotics can also interact with the host via metabolites, including SCFAs and vitamins, that interact with the host. These metabolites exhibit antimicrobial activities, as demonstrated by cell-free supernatants derived from *L. reuteri* (Yang et al., 2021). These metabolites have demonstrated the ability to inhibit pathogenic bacterial growth, reduce liver injury, and modulate inflammation in various cell models, highlighting the extensive potential of probiotic metabolites (Yang et al., 2021).

Probiotics and postbiotics are versatile feedstuffs with many beneficial characteristics; it is also important to understand the different mechanisms impacting the GIT.

1.6.2. Modes of Actions of Probiotics in Pigs

Competitive exclusion is a phenomenon where the normal microbiota in the gut protects against harmful microorganisms, reducing the risk of intestinal infections in pigs (Yirga, 2015). Studies have shown that *Lactobacilli* strains can successfully inhibit the growth of *E. coli* and can inhibit *E. coli* attachment to the small intestinal epithelia in piglets (Yirga, 2015). Other research has indicated that the administration of certain probiotics like *Pediococcus acidilactici or Saccharomyces cerevisiae boulardii* can limit the attachment of *E. coli* to the ileal mucosa, a crucial step in the pathogenesis of the pathogen (Yirga, 2015).

Competitive exclusion involves selected beneficial microorganisms competing with harmful ones for adhesion sites and organic substrates in the gut. Probiotic microorganisms adhering to the gastrointestinal wall can prevent the colonization of pathogenic microorganisms, blocking receptor sites against pathogen attachment (Liao et al., 2017). This process increases beneficial microbial colonization, inhibiting the adhesion of harmful bacteria to the intestinal epithelia and, consequently, blocking receptor sites for pathogen attachment and hence, prevent infections by excluding harmful pathogens from establishing themselves in the gut (Liao et al., 2017).

The competitive exclusion concept also implies that probiotics compete with pathogenic bacteria for nutrients and absorption sites within the gut (Yang et al., 2015). This competition, primarily for energy and nutrients like carbon sources, can suppress the growth of pathogenic bacteria. While the gut is abundantly rich in nutrients, it is important to recognize that microbial growth can be inhibited in an environment deficient in even a single essential nutrient. Additionally, the rapid utilization of energy sources by probiotics may shorten the bacterial growth log phase,

making it challenging for bacteria to withstand the flushing effect caused by gut peristalsis (Yirga, 2015).

Once established in the gastrointestinal tract, certain probiotic microorganisms generate substances with bactericidal or bacteriostatic properties, agents that prevent the growth of bacteria (Bajagai et al., 2016). These substances can inhibit the colonization of the host intestine by unwanted microorganisms, including both gram-positive and gram-negative bacteria. This microbial antagonism action counters the disturbance of the host gut microbial balance caused by harmful microbes, promoting a favorable eubiotic (mixture of probiotics and prebiotics) status (Bajagai et al., 2016).

Many probiotic bacteria, particularly LAB, ferment carbohydrates such as lactose, producing SCFAs such as lactic and acetic acids. This process lowers the luminal pH to a level that is often not tolerable by harmful bacteria (Bajagai et al., 2016). Some species also produce hydrogen peroxide, inhibiting the growth of gram-negative bacteria (Bajagai et al., 2016). The reduction in gut pH by these substances may partially compensate for the low secretion of hydrochloric acid in the stomach of weanling piglets (Yirga, 2015).

In addition to organic acids, probiotic bacteria can produce various other substances, including antioxidants, antimicrobial peptides (i.e. defensins), reuterin, bacteriocins, and microcins. These substances reduce the number of viable pathogenic organisms and influence bacterial metabolism and toxin production (Hou et al., 2015). Lactic acid bacteria-produced bacteriocins have been reported to penetrate the outer membrane of gram-negative bacteria, exhibiting antimicrobial activity by reducing harmful bacteria growth.(Alakomi et al., 2000).

Microcin, produced by probiotic *E. coli*, can restrict the growth of other competing bacteria in an inflamed intestine, including commensal *E. coli*, adherent-invasive E. coli, and the related pathogen *S. enterica* (Sassone-Corsi et al., 2016).

The gastrointestinal lumen contains beneficial nutrients and microorganisms and harmful substances like toxic materials and foreign antigens (Willing et al., 2012). The epithelial cells on the GIT mucosa form a permeable barrier that acts as the initial defense against harmful microbes in the GIT. However, stress or disease conditions can disrupt this barrier (Willing et al., 2012).

Probiotics can restore the barrier function of the GIT mucosa in both in vitro and in vivo models (Madsen et al., 2001). They can also influence interactions between intestinal mucosal cells and enhance cellular stability by modulating the phosphorylation of cytoskeletal and TJ proteins (Willing et al., 2012). This action is related to changes in mucus or chloride secretion and alterations in epithelial cells' expression of TJ proteins (Yang et al., 2015).

L. plantarum was demonstrated to protect epithelial cells against damage induced by enterotoxigenic *E. coli* by diminishing the upregulation of interleukin 8 (IL-8) and tumor necrosis factor α (TNF-α) gene expression (Wu et al., 2016). These immune compounds are pro-inflammatory cytokines that play a key role in the recruitment and activation of neutrophils during inflammation (French et al., 2018). This protection was achieved by maintaining the gene expression and other contents of critical TJ proteins (Wu et al., 2016).

The host's recognition of pathogenic, commensal, and probiotic bacteria and the resulting immune responses involve bacterial macromolecules called microbe-associated molecular patterns. Studies have highlighted the key role of microbe-associated molecular patterns in the communication between beneficial

microorganisms and the host. These microbe-associated molecular patterns interact with pattern recognition receptors in the host GIT mucosa, including toll-like receptors. The signaling interactions between the innate pattern recognition receptors and the microbe-associated molecular patterns of probiotics contribute to stabilizing host mucosal immunity (Maldonado et al., 2015).

Probiotics also have the capacity to impact the host immune system through various products such as metabolites, cell wall components, and deoxyribonucleic acid (DNA). The immunostimulatory effects of probiotics have also been observed in mice, where a significant increase in gut innate immune cells such as macrophages and dendritic cells was observed following oral administration of *L. casei* CRL 43 (Galdeano et al., 2006). Probiotics can also affect the immune system by enhancing cell-mediated immunity, which is a protective mechanism that is not generated by antibodies, as well as the stimulation of T-cell migration (Azizi et al., 2022). Exposure to viable probiotic bacteria or bacterium-derived components can trigger the acquired immune system, leading to improved interaction of leukocytes and elimination of potential pathogens (Hughes & Heritage, 2002).

Diarrhea poses a significant challenge for pigs in the initial weeks post-wean. Around 80% of reported studies have observed decreased diarrhea occurrence in pigs receiving probiotics (Simon, 2005). Taras et al. (2006) found that long-term application of *E. faecium* reduced post-wean diarrhea and overall pre-wean mortality. Other studies, such as Bhandari et al. (2010), demonstrated reduced diarrhea incidence by adding specific probiotics or probiotic combinations.

The promotion of favorable GIT microbiota through probiotics contributes to a reduction in gut pathogenic infections and diarrhea incidence in swine. This reduction

implies fewer veterinary interventions, potentially saving costs for swine producers, and enhances overall production efficiency (Simon, 2005).

Numerous studies have shown that probiotics can enhance the digestibility of dietary nutrients. For instance, Huang et al. (2004) reported increased digestibility of CP and P of a diet supplemented with a complex *Lactobacilli* preparation fed to weaned pigs. Yu et al. (2008) demonstrated that *L. fermentum* maximized dietary CP digestibility in weaned pigs, while Meng et al. (2010) found improved CP and energy digestibility in diets containing probiotics when fed to growing-finishing pigs. Giang et al. (2010; 2012) observed increased ileal and total tract digestibility of organic matter, CP, and crude fiber in diets supplemented with various LAB complexes when fed to weaned pigs.

The improved digestibility attributed to probiotics may result from increased production and activity of digestive enzymes in the gut, as probiotics exhibit high fermentative activity (Upadhaya et al., 2015). *Lactobacilli*, for example, produces lactic acid and proteolytic enzymes that enhance nutrient digestion in the GIT (Yu et al., 2008). Studies have reported increased sucrase, lactase, and tri-peptidase activities in response to probiotics in pre-weaned piglets (Collington et al., 1990). Additionally, certain probiotics, such as *Lactobacillus* sp. and *B. amyloliquefaciens* have produced active enzymes, including amylase, lipase, phytase, and protease.

Probiotics may also impact the absorption and secretion activities of the swine GIT. Kenny et al. (2011) observed higher L-glutamine transport and increased ion secretion in pigs treated with *B. cereus* or *E. faecium*. Cai et al. (2015) reported longer intestinal villi in pigs fed a DFM product, indicating increased nutrient absorptive surface.

1.6.3. Current Understanding of Postbiotic Mechanisms of Action

Certain metabolites in postbiotics possess antioxidant properties, enhancing animal health and production performance. A key example is β-glucan, a polysaccharide found in fungal cell walls that searches for hydroxyl radicals and singlet oxygen, protecting DNA from oxidative damage (Pourahmad et al., 2011). Additionally, some bacteria, LAB, produce exopolysaccharides, lipoteichoic acid, and cell-surface proteins, which are present in postbiotics and exhibit antioxidative functions within the host (Zhao et al., 2024). Postbiotics derived from *L. plantarum* have also been shown to impact malondialdehyde levels in the serum of post-weaned lambs (Bai et al., 2016). Measuring malondialdehyde level is commonly known as a marker of oxidative stress and antioxidant status (Gawel et al., 2004). Mechanistic pathways involved in the antioxidant properties of postbiotics include the inhibition of the production of reactive oxygen species induced by stressors, supplementation of antioxidants for the host, and improved antioxidant enzyme gene expression and activity (Zhao et al., 2024).

Postbiotics retain structural components that boost the host's immune function even after deactivating the source organism. For example, TLR2 is linked to the inflammatory response of Gram-positive bacteria, activated by bacterial compounds like lipoproteins, lipoteichoic acid, and peptidoglycan (Zhao et al., 2024). The yeast cell wall, rich in mannan, β-glucan, and chitin, affects immune function and host-pathogen interactions. β-glucan, found in the cell wall of *S. cerevisiae*, supports structure and stimulates innate and adaptive immune responses (Qi et al., 2011).

Postbiotics, derived from the metabolic processes of probiotics, are known for their beneficial effects on the host microbiota. These benefits are delivered through

three primary mechanisms: the presence of antimicrobial substances, the modulation of the gut environment, and the incorporation of structural components that promote the adhesion of beneficial microbes (Zhao et al., 2024). Postbiotics contain antimicrobial substances such as bacteriocins, which are peptides that inhibit the growth of pathogenic bacteria. These bacteriocins are highly effective in targeting other bacteria, providing a defense mechanism within the gut. Postbiotics can modulate the gut environment by introducing organic acids, influencing pH levels, and establishing adequate conditions for the growth of beneficial bacteria while reducing the proliferation of pathogens (Thu et al., 2011). Postbiotics encompass structural components such as fimbriae and lectins, which enhance the adhesion of beneficial microbes to specific sites within the gut. This adhesion is a key factor in establishing and maintaining beneficial microbial populations (Lebeer et al., 2012).

One of the mechanisms of maintaining gut barrier function through postbiotics is through enhancing Mucin-2 expression. This has been demonstrated in mouse models, where the supplementation of *L. paracasei* increased mucin-2 production (Wei et al., 2023). Mucin is secreted by goblet cells and plays a pivotal role in maintaining gut barrier function (Wei et al., 2023).

1.6.4. Gut Physiological Responses with Probiotics

1.6.4.1. Improvements in Villi Height and Crypt Depth

The small intestine's structure and the intestinal barrier's integrity play a crucial role in the digestion, absorption, transport of nutrients, and the reduction of pathogenic particle entering the body. The intestinal epithelial cells, including enterocytes, goblet cells, and Paneth cells, play a vital role in nutrient absorption, immunoglobulin secretion, antimicrobial peptide synthesis, and maintaining intestinal homeostasis through interactions that impact gut health (Szabó et al., 2023). The

relationship between nutrient assimilation and the morphological characteristics of the intestine, such as VH and the ratio of VH to CD, has been well-established. Reduced VH and increased CD are associated with nutrient malabsorption, heightened gastric secretion, and diarrhea (Zhang et al., 2023). Interventions with beneficial microorganisms, such as *L. plantarum* and *B. subtilis*, have positively affected intestinal morphology. For instance, *Lactobacillus plantarum* mitigated a decrease in jejunal VH caused by enterotoxigenic *E. coli*, while *B. subtilis* increased VH and the VCR in the ileum, expanding nutrient absorption areas and enhancing digestion and absorption in weaned piglets (Zhang et al., 2023)

As noted earlier, maintaining TJs between intestinal epithelial cells is crucial for the integrity of the intestinal mucosal barrier. Damage to TJs increases cell permeability, allowing bacteria and pathogens to penetrate the mucosa and potentially cause diseases. Probiotics have been shown to enhance intestinal TJs, protecting intestinal epithelial cells against pathogenic invasion. For example, *L. plantarum* prevents the adhesion of enterotoxin-producing *E. coli* to intestinal epithelial cells, ensuring the integrity of the intestinal barrier (Zhang et al., 2023).

1.6.5. Importance of Lactobacillus and Bifidobacteria in Pig Performance

The most frequently used commercial probiotics are *Lactobacillus spp.*, *Bifidobacterium spp.*, *Streptococcus spp.*, and yeasts (Patil et al., 2015). Supplementing the microbiome of piglets with probiotic bacteria such as *Lactobacillus spp.* and *Bifidobacterium spp.* probiotics could help create an optimized microbiome by improving the abundance and number of *Lactobacilli spp.* and other indigenous probiotic bacteria (Yang et al., 2017). Therefore, promoting the growth of the weaned pig by improving intestinal development, enhancing antioxidant capacity,

and modulating gut microbiota (Pang et al., 2022). Vigors et al. (2016) observed a positive connection between efficient feed utilization in pigs and an increased presence of *Lactobacillus spp.* in the cecum. From a physiological standpoint, improved feed efficiency is associated with better digestion and nutrient absorption in the intestines (Baird, 1977).

When fiber-fermenting *Lactobacilli* are present in the gut, there is an increase in acetate and butyrate concentration. These compounds serve as an energy source for colonic cells, as discovered by Heinritz et al. (2016). Additionally, greater acetate and butyrate in the large intestine contributes to favorable pig growth performance (Hou et al., 2015). *Lactobacillus* strains also produce active dietary enzymes like amylase, lipase, phytase, and protease, which play a crucial role in digesting and absorbing nutrients in the intestines, as highlighted by Kim et al. (2007).

An inadequate immune response can adversely affect feed utilization efficiency and pigs' daily weight gain. This is because immune responses come with a metabolic cost for the host, necessitating compromises in other energy-demanding biological processes like growth, reproduction, and thermoregulation (Rauw, 2012). In the presence of an actively unregulated immune response, the excessive production of proinflammatory cytokines and inflammatory mediators can compromise the integrity and function of the epithelium, leading to disruptions in nutrient transport across the intestinal surface (McKay & Baird, 1999). Therefore, reducing inflammatory markers due to greater *Lactobacilli* concentration in the GIT is crucial for maintaining gut health (Liu et al., 2014).

Regarding complex probiotics containing *Lactobacilli*, the elevated presence in the gastrointestinal tract could potentially enhance the activity of beneficial enzymes like β-galactosidase, positively impacting nutrient utilization (Fuller, 2012).

Bifidobacterium strains are commonly used in the livestock industry to generate probiotics and postbiotics. Studies have demonstrated the benefits of including *Bifidobacterium*-based postbiotics in weaned pigs (Zhao et al., 2024). *Bifidobacterium* is a genus of anaerobic, gram-positive microorganisms that do not produce gas and form spores (Zhao et al., 2024). These bacteria are also catalase-negative, which plays a key role in the defense against oxidative stress (Yuan et al., 2021). Like LAB, *Bifidobacteria* ferment glucose into lactic and acetic acids. However, they differ from LAB by having the enzyme fructose-6-phosphate phosphoketolase (Zhao et al., 2024).

Barba-Vidal et al. (2017) reported a study evaluating a combination of two probiotic strains, *B. longum* subsp. *infantis* and *B. animalis* subsp. lactis BPL6, in promoting gut health and mitigating the impact of a *Salmonella* challenge in weaning piglets. Results showed that the probiotic combination improved feed intake, reduced *Salmonella* excretion, lowered rectal temperature, and enhanced the VCR in *Salmonella*-challenged animals. Additionally, inclusion of the probiotic combination reduced diarrhea incidences, increased intestinal immune response, and an improved VCR, which was observed in both challenged and non-challenged groups (Barba-Vidal et al., 2017).

Pang et al. (2022) demonstrated that supplementing weaned pigs with *B. animalis* (10^{10} CFU per kg in the diet). Average daily gain was increased in pigs fed *B.animalis* during day 15 to 28. He also showed improved results in intestinal morphology while supplementing *B. animalis*. Pigs fed with the dietary treatment

improved the VH and VCR in the duodenum. Additionally, the supplementation of *B. animalis* increased the jejunal goblet cell numbers. The same study by Pang et al. (2022) also evaluated enzyme activity, where results showed that supplementing *B. animalis* increased amylase activity in the jejunum.

1.7. Conclusions

Weaning is an important phase of a pig's life cycle, where the piglet encounters various stressors as soon as it is separated from the sow. These stressors have major impacts on the pigs' health. Therefore, a phased-feeding strategy in the nursery is needed to gradually transition the young pig from consuming a high-fat, high-lactose, liquid milk diet before weaning to consuming a low-fat, low-lactose, high-carbohydrate, dry diet comprised of cereal grains and soybean meal. Meeting the nutritional requirements early is essential for GIT development, bone and muscle growth, and decreased morbidity. Feed additives such as probiotics and postbiotics influence feed intake and gut health. The role of *Lactobacillus* and *Bifidobacteria*-based probiotics and postbiotics benefit the weaned pigs' growth performance, immune system, and intestinal microbiota, which aid the newly weaned pig in overcoming health challenges.

1.8. Research Objectives

This thesis work evaluated 2 dietary strategies (i.e. use of a novel protein ingredient and probiotic and postbiotic supplementation) to aid the weaned pig during the transition phase. Both research trials include evaluation of pig growth performance due to the economic importance of growth to overall production efficiency and assessment of intestinal health as a means to understand expected differences in performance.

Study 1 evaluated increasing levels of CFP (4%, 8%, and 12%) as a substitute for SPC and its effects on growth performance and gut permeability in nursery pigs. It was hypothesized that the inclusion of CFP would replace SPC without compromising growth performance and gut permeability.

Study 2 elucidated the potential of *Lactobacillus*-based probiotic and *Bifidobacteria*-based postbiotic inclusion in nursery diets on growth performance from weaning to market, alterations in microbial populations, intestinal histology, and carcass value. It was hypothesized that probiotic and postbiotic inclusion would positively influence microbiome composition, intestinal morphology, and ultimately, growth performance.

EVALUATING THE EFFECTS OF REPLACING SOY PROTEIN CONCENTRATE WITH A NOVEL CORN FERMENTED PROTEIN ON GROWTH PERFORMANCE AND GUT INTEGRITY OF NURSERY PIGS

2.1. ABSTRACT

Corn fermented protein (CFP) is derived from the production of dry-mill bioethanol. This novel product may replace high quality proteins in the diet because CFP is an excellent source of lysine and methionine and can have up to 50% CP and contains 25% yeast. Benefits of CFP due to the fermentation process include improved CP digestibility, increased energy digestibility, and a reduction in crude fiber (CF) levels compared to non-fermented protein sources. Previous research done with CFP using shrimp suggests that an 18% inclusion of CFP to replace soybean meal (SBM) and fish meal (FM) can be utilized without affecting growth performance. This study also showed that the yeast portion of CFP (*Saccharomyces cerevisiae*) helps enhance disease resistance and is an excellent source of essential amino acids (AA). Research on CFP in pigs has also shown that it contains a greater concentration of digestible AA and metabolizable energy (ME) than low-oil distillers dried grains with solubles (DDGS) (Stas et al., 2022). The current study aimed to determine the effects of increasing dietary CFP inclusion, replacing soy protein concentrate (SPC) on weight gain, feed intake, feed efficiency, and gut integrity of weaned pigs. In total, 1144 pigs were distributed evenly into 44 pens (13 barrows and 13 gilts/pen; initial body weight (BW) 6.0 ± 0.1 kg), with 286 pigs assigned per treatment and 11 replications. The four treatments were designed as a titration of CFP inclusion at 0%, 4%, 8%, and 12% in Phase 1 (3.62 kg/pig feed budget) and 0%, 2%, 4%, and 6% in Phase 2 (5.44 kg/pig feed budget) replacing SPC. Pigs were fed a common diet through Phase 3 (10.28 kg/pig feed budget). A differential sugar

absorption test (DSAT) was administered using a 5% lactulose and 5% mannitol solution on the tenth day to pigs consuming the 0% and 12% CFP diets to determine gut integrity. Urine was collected to measure differences in sugar ratios to assess gut permeability. Similar average daily gain (ADG) responses of pigs fed the 4% and 8% CFP inclusion compared to pigs fed the 0% CFP diet occurred during Phase 1 ($P<0.01$). Pigs fed diets with 4% and 8% CFP inclusion in the second week of Phase 1 had greater average daily feed intake (ADFI) than pigs fed 12% CFP diets ($P<0.01$); pigs fed the 0% CFP diet had an intermediate response. At the end of Phase 2, pigs fed diets with 0%, 2%, and 4% CFP had greater ADFI than pigs fed the diet with 6% CFP ($P<0.01$). After the first week of Phase 2, pigs fed 0%, 2%, and 4% inclusions of CFP had greater G:F than pigs fed 6% CFP ($P<0.01$). There was no statistical difference between the 0% and 12% inclusion of CFP when subjected to a DSAT test, suggesting that there was no reduction in gut integrity when including CFP in the diet. Replacing SPC with CFP up to 8% in the diet for the first two weeks post-wean and 4% for the next two weeks in the nursery can be effective based on improved ADFI and similar G:F.

Keywords: Corn fermented protein, Growth Performance, Gut Integrity, Nursery Diets

2.2. INTRODUCTION

Weaned pigs, particularly during the first-week post-wean, undergo significant biological stress when transitioning from the sow. This stress can lead to intestinal and immune system dysfunctions, reducing health, growth, and feed intake. The abrupt change from liquid milk to a dry cereal-based diet is a key physiological stressor a nursery pig experiences (Pluske, 2016). ‘Complex’ nursery diets contain multiple sources of energy and protein to encourage feed intake and adequate nutrition in

newly weaned pigs. Improved feed intake leads to lower intestinal disturbances (e.g., reduced enzyme activity and reduced nutrient absorption) and lower occurrence of gastrointestinal tract (GIT) inflammation and diarrhea (Weasley et al., 2021) which help to mitigate increased intestinal permeability and inflammation and lower body weight (BW) gain associated with weaning (Fabá et al., 2023).

A complex nursery pig diet typically includes highly digestible specialty ingredients to provide high-quality nutrition and stimulate feed intake in the early post-wean period (Menegat et al., 2019). Newly weaned pigs can easily digest lactose and proteins similar to those found in milk but have limited ability to digest plant proteins and utilize fat. Pigs also have a hypersensitivity reaction to soybean meal (SBM) induced by allergenic proteins and indigestible carbohydrates of soybeans (Menegat et al., 2019).

Soybean meal (SBM) contributes high-quality protein diets for livestock due to its rich limiting AA content and are a significant source of energy for pigs. However, providing SBM in cereal-based diets for weaned pigs may impair health by triggering a hypersensitive immune response in the GIT (Stas et al., 2022). This immune response, caused by specific antigenic proteins like glycinin and β-conglycinin, leads to structural changes in the small intestine, damaging the microvilli, and reduces nutrient absorption capacity (Goebel & Stein, 2011). To address this, enzymatically treated SBM is sometimes used due to its lower anti-nutritional factor content (Li et al., 2021). However, this approach can increase diet expenses, and its impact on the growth performance of weanling pigs is inconsistent. Another alternative high quality protein source is soy protein concentrate (SPC).

Soy protein concentrate is a highly concentrated protein product derived from soybeans that have been dehulled and de-oiled. Soy protein concentrate typically contains at least 65% crude protein (CP) (Menegat et al., 2019). During SPC production, allergenic proteins and indigestible carbohydrates from soybeans are largely eliminated. However, the antinutritional factor trypsin inhibitor may be present in higher quantities compared to SBM because processing methods often do not involve heat treatment (Menegat et al., 2019). In nursery diets, incorporating around 14% SPC has been shown to enhance growth performance compared to SBM. However, higher inclusion rates may negatively impact palatability and reduce feed intake and the cost of SPC typically makes it impractical for nursery diets (Menegat et al., 2019). Alternative feedstuffs, such as corn fermented protein (CFP), appears as a viable option to support the growth and development of the newly weaned pig. Improved methods in starch-to-ethanol conversion have revolutionized ethanol production, paralleling advancements in wet milling and oilseed processing industries. These innovations concentrate nutrients in co-products, elevating their nutritional value for animal feed. Notably, technologies like separating corn fiber pre- or post-fermentation, concentrating protein and yeast, and removing varying amounts of corn oil have given rise to new corn co-products, such as CFP (U.S. Grains Council, 2023). Corn fermented protein is produced when protein and yeast fractions of ethanol production are added back to high protein dried distillers grains with solubles (HPDDGS) (Stas et al., 2022). Corn-fermented protein contains 50% CP, while conventional DDGS contains 25-35% CP (Garavito-Duarte et al., 2024). Corn-fermented protein also has greater digestible energy (DE) and metabolizable energy (ME) than corn and DDGS, according to Stein et al. (2023). Corn fermented protein also contains greater yeast concentrations, contributing to a better amino acid (AA)

profile compared to SBM and fish meal (Garavito-Duarte et al., 2024). Corn fermented protein has also been demonstrated as an effective ingredient in diets for turkeys, aquaculture, and the pet food industry up to 20% inclusion in the diets (Scholey et al., 2024).

Therefore, this study aimed to evaluate the effects of graded levels of CFP to replace SPC on BW, average daily gain (ADG), average daily feed intake (ADFI), gain to feed ratio (G:F) and GIT integrity in nursery pigs.

2.3. MATERIALS AND METHODS

2.3.1.Animals, Housing and Feeding

A total of 1,144 pigs (initial BW 6.1 ± 0.1 kg) were selected for this specific study from a population of 1,240 pigs (PIC 800 x PIC) delivered to the South Dakota State University Off-site commercial swine wean-to-finish research facility. Pigs were allocated to 44 pens, (20 pens in the east barn and 24 pens in the west barn; each treatment was represented in each of the barn's section), blocked by weight and barn location to have 26 pigs per pen (13 barrows and 13 gilts/pen; n=11 pens/treatment). Each pen provided an area of 3.1 m x 6.9 m (approximately 0.82 m^2 per pig). Pens were assigned to one of 4 dietary treatments with increasing levels of CFP, replacing SPC provided within 2 diet phases. For Phase 1 (day 0 to 14), the inclusion of CFP was: 1) 0%, 2) 4%, 3) 8%, 4) 12% (Table 2.1). For Phase 2 (day 14 to 28), the percentage of CFP was reduced (0%, 2%, 4%, and 6%) to match changes in pig nutritional requirements with age as per NRC (2012) (Table 2.2). Phase 3 (day 28 to 42), all pigs were provided with a common diet (Table 2.3). The facility was equipped with a Feedlogic M-Series (Feedlogic ComDel Innovation, Wilmar, MN) system for feeding. Feed delivery to pens started with two cycles per day (i.e., every twelve

hours), followed by four cycles per day (i.e., every six hours). Pigs in this study were provided with a total of 3.63 kg/pig feed budget for Phase 1, 5.44 kg/pig feed budget for Phase 2, and 10.28 kg/pig feed budget for Phase 3. Growth performance parameters (BW, ADG, ADFI, and G:F) were evaluated until day 42.

2.3.2.Experimental Design

The experimental design used for this study was a randomized complete block design with 11 blocks, defined as location within the room, containing each treatment (five blocks in one the room and six blocks in the other room) with treatments randomized within each block.

2.3.3.Feed Measurements and Calculations

All pens contained one five-hole dry feeder and two cup waterers for *ad libitum* access to feed and water, respectively. Feed remaining on weigh day was calculated according to a prepared calibration curve by measuring the distance from the top of the feeder to the top of the leveled feed and its density, as previously described (Clizer et al., 2022). The feed disappearance was calculated using the drop history recorded by the Feedlogic program (i.e., the total feed delivered from the previous weigh day to the relevant weigh day) and subtracting the feed remaining on the relevant weigh day.

2.3.4.Target Nutrition levels

Nutrient requirement targets were set to meet or exceed the Nutrient Requirements of Swine 12th edition recommendations (NRC, 2012). The ME requirements for pigs of a body weight range of 5-7 kg and 7-11 kg require an effective ME content of 3,400 kcal/kg. In Table 2.3 and Table 2.6 we describe the ME and AA targets for Phases 1 through 3. According to Table 2.2, leucine levels in CFP diets were higher in its

content relative to the 0% CFP diets. The CFP diets were formulated using the nutrient profile information from Acosta et al. (2021).

2.3.5.Pen Weights and Growth Performance Calculations

Pens of pigs were weighed at barn entry and then at weekly intervals, with day 42 marking the end of the trial. Growth performance parameters of BW, ADG, ADFI, and G:F were calculated by a series of formulas related to pig day as noted below. Pig day includes the removal date(s) and weight(s) of any pig(s) between weigh day without compromising the calculation of performance results. Specifically, pig day account for the day from the last weigh day to the current, multiplied by the number of pigs in that particular pen on the weigh day, and lastly, the number of day any removed pigs were on trial and the weight of any pigs on the day of removal were added.

Growth performance calculations:

$$ADG = (current\ pen\ weight - previous\ pen\ weight + removed\ pig\ weights) \div pig\ days$$

$$ADFI = (feed\ delivery - feed\ WB) \div pig\ days$$

$$G:F = ADG \div ADFI$$

2.3.6.Differential Sugar Absorption Test

On day 10 of this study, representative pigs fed 0% CFP and pigs fed 12% CFP were subjected to a differential sugar absorption test (DSAT) to assess intestinal permeability (Wijtten et al., 2011). Specifically, 11 pigs per treatment were randomly selected from the pen to be evaluated. Pigs were randomly assigned to one of nine individual cages (0.56 × 0.64 × 0.89 m^2) with access to Phase 1 feed and water (provided in a bowl). Due to cage availability, 9 pigs were evaluated in the first 6

hours, 9 pigs were evaluated during the next 6 hours, and 4 pigs were evaluated 6 hours after that. They were then orally administered a bolus containing 5% lactulose (L) and mannitol (M) at 15 mL/kg using a syringe and liquid feeding tube, followed by total urine collection for 6 hours (Perez-Palencia et al., 2021). After this period, the pigs were returned to their original pens. A subsample of urine was homogenized and stored at -80°C for later analysis of the lactulose to mannitol ratio (L:M) using the EnzyChrom Intestinal Permeability Assay Kit (Catalog No: EIPM-100, BioAssay Systems, Hayward, CA) as an indicator of intestinal permeability (Hong et al. 2020).

2.4. STATISTICAL ANALYSIS

2.4.1.Growth Performance

The BW, ADG, ADFI, and G:F of the nursery pigs were subject to linear mix model using the PROC GLIMMIX procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC), considering the main effect of dietary treatment as a fixed effect and the pen as the experimental unit. Any statistical differences between treatments was considered with a *P*-value ≤0.05. A trend for differences between treatments was considered with a *P*-value of <0.10 and >0.05.

2.4.2.Differential Sugar Absorption Test

The PROC UNIVARIATE of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) was used to analyze the DSAT results. Tukey's adjusted means test was used to detect differences between treatment groups where the main effect of treatment was significant. Significance was considered at $P \leq 0.05$.

2.5. RESULTS

Throughout the feeding period from day 0 to day 42, there were no significant differences ($P \geq 0.27$) in BW among nursery pigs because of diets with increasing inclusions of CFP to replace SPC (Table 2.3). In the first week (day 0 to 7), pigs fed diets with 12% CFP inclusion had significantly lower ($P = 0.02$) ADG than the rest of the dietary treatments (Table 2.4). Performance in week 2 (day 7 to 14) showed similar findings, where pigs fed diets with 8% and 12% CFP inclusion tended ($P = 0.09$) to have lower ADG than pigs fed diets with 0% and 4% CFP inclusion. There were no significant differences ($P \geq 0.15$) in ADG among treatments for the next 4 weeks up to and including the end of the study. By phase, the ADG of pigs fed the highest CFP inclusion was lower ($P < 0.01$) than all other treatment groups in Phase 1 and there were no significant differences ($P \geq 0.26$) between treatments in ADG in Phase 2 and 3. The ADFI in the first week tended to be lower ($P = 0.06$) in pigs fed diets with 12% CFP than pigs fed 0% and 4% CFP, while pigs fed diets with 8% CFP showed intermediate ADFI. For the second week of supplementation (day 7 to 14), pigs fed diets with 4% and 8% CFP inclusion recorded greater ($P < 0.01$) ADFI than pigs fed diets with 12% CFP inclusion; pigs fed diets with 0% CFP has intermediate ADFI. Furthermore, ADFI showed that pigs fed diets with 4% and 8% CFP inclusion consumed more ($P < 0.01$) feed than pigs fed diets with 12% CFP in Phase 1. For Phases 2 and 3, there were no significant differences in ADFI across treatments. Pigs fed diets with 0%, 4%, and 8% CFP inclusion had a greater ($P < 0.01$) G:F than pigs fed diets with 12% CFP inclusion at the end of week one (day 0 to 7; Table 2.6). There were no differences ($P = 0.32$) in week 2 (day 7 to 14) for G:F. For week 3 (day 14 to 21) and week 4 (day 21 to 28), pigs fed diets with 12% CFP inclusion had higher ($P < 0.01$) and lower ($P < 0.01$) G:F than the rest of the treatment groups,

respectively. Pigs fed diets with 0% CFP inclusion had a lower ($P = 0.03$) G:F than pigs fed diets with 8% and 12% CFP inclusion in Phase 1. There were no differences ($P \geq 0.85$) in G:F in Phase 2 (day 14 to 28) and when pigs were fed a common diet (day 28 to 42). For the DSAT, there were no differences ($P \geq 0.25$) between sugar absorption by pigs fed 0% CFP inclusion and pigs fed 12% CFP inclusion.

2.6. DISCUSSION

Results from this study showed no differences in BW when including increasing levels of CFP to replace SPC. These findings are similar to those from Stas et al. (2021), who evaluated the effects of two levels of CFP (5% and 10%) versus two levels of enzymatically treated SBM (5% and 10%) on nursery pigs, where BW was unaffected by levels of inclusion during the thirty-one-day period. However, Garavito-Duarte (2024) reported lower BW at days 21 and 42 after weaning in pigs fed diets containing 15% CFP compared to diets containing fishmeal and enzymatically treated SBM. The inclusion of corn co-products in nursery pig diets may disrupt the balance of branched-chain amino acids (BCAA), leading to decreased growth performance. Research by Kwon et al. (2022) indicated that increasing the standardized ileal digestible leucine to lysine ratio reduced ADG and ADFI in growing pigs, 9.9 kg pigs. Cemin et al. (2019) suggested that higher levels of isoleucine and valine could counteract the negative effects of excess leucine on pig growth. Hong et al. (2023) reported that including high levels of DDGS (30%) in growing-finishing diets with SID BCAA:Lys (78% Val:Lys, 70% Ile:Lys, and 160% Lsu:Lys) improved growth performance.

This study's ADFI results showed that pigs fed 4% and 8% CFP inclusion, replacing SPC, consumed more feed daily than pigs fed 12% during day 0 to 7. Results from Stas et al. (2021), differed from our findings, where they reported no

significant differences regarding ADFI from Day 0 to 10 when evaluating two levels of CFP (5% and 10%). The findings of Garavito-Duarte et al. (2024) cannot be compared to this study because of different feedstuffs; however, pigs fed a diet with CFP plus added yeast mass had lower ADFI at day 14 compared to pigs fed fish meal and or enzymatically treated SBM. The importance of greater feed intake in the first six days post-wean, as observed in this study, is the association with greater development of the GIT and a reduction in protein fermentation in the small intestine which can positively impact overall health status of the nursery pig (Fabá et al., 2024).

Adding CFP, up to a 12% inclusion rate during the first two weeks after weaning, decreased G: F. These results reflect less efficient use of feed in converting feed into body weight gain. Feed efficiency represents the cumulative efficiency with which the pig utilizes dietary nutrients for maintenance, lean gain, and lipid accretion. It is closely linked with energy metabolism, as the oxidation of carbon-containing components in the feed drives all metabolic processes (Patience et al., 2015).

The lack of difference in the DSAT ratio indicated that inclusion 12% CFP had no apparent impact on intestinal permeability. Similarly, Garavito-Duarte et al. (2024), reported no difference in intestinal permeability in nursery pigs fed with a CFP plus added yeast mass. It is normal for increased intestinal permeability in weaned piglets to begin approximately 24 hours after weaning, with gradual recovery typically occurring by the second week in the nursery (Moeser et al., 2007). The DSAT test assesses intestinal permeability by measuring the ability of two unmetabolized sugar molecules, lactulose and mannitol, to pass through the intestinal mucosa (Wijtten et al. 2011). Lactulose, being relatively larger than mannitol, enters the bloodstream primarily through the paracellular route or due to damage at the tight

junction barrier in the intestinal epithelium, which permits the penetration of larger molecules (Vojdani 2013). Mannitol has been demonstrated to be an undigestible carbohydrate, which the majority reaches the large intestine (Maekawa et al., 2009). An often-overlooked limitation of the conventional understanding of the DSAT test is that mannitol is not significantly absorbed through a transcellular process. Furthermore, this interpretation of the lactulose test does not align with the current knowledge of paracellular pathways, which include three types: pore, leak, and unrestricted (Ordiz et al., 2018). Nevertheless, the dual-sugar method is advantageous due to its low cost, minimal invasiveness, and practicality for regular livestock screening (Sujiyanto et al., 2024). Therefore, we can conclude that the inclusion of CFP at the highest level in this experiment (12%) did not impact GIT permeability.

2.7. CONCLUSION

Providing graded levels of CFP in nursery diets to replace SPC did not influence the body weight gain of newly weaned pigs through the nursery period; however, when CFP was included at 12%, G: F in the first weeks was decreased, meaning a less efficient use of nutrients. Although growth performance was negatively impacted by the highest CFP inclusion level in this study, no evidence of any negative impact on GIT health was noted based on the lack of difference in intestinal permeability. Therefore, CFP can be a suitable feedstuff for nursery pigs.

Table 2.1 Dietary Phase 1 ingredient composition (kg).

Phase 1				
	CFP 0%	**CFP 4%**	**CFP 8%**	**CFP 12%**
Corn	275	274	272	268
Dried Whey	200	200	200	200
Steamed rolled Oats	200	200	200	200
Soybean Meal	150	150	150	150
Soy Protein Concentrate	120	80	40	0
Corn Fermented Protein	0	40	80	120
Soy Oil	20	20	20	20
Phosphate 21%	11	11	11	11
Limestone	10	10	10	10
Salt	5.0	5.0	5.0	5.0
L Lysine 98%	3.7	5.1	6.6	8.0
DL Methionine	2.2	2.4	2.9	3.3
L Threonine	1.0	1.4	1.5	2.5
Vitamin-Mineral Mix[1]	1.8	1.8	1.8	1.8
Total	1000	1000	1000	1000

[1]Provided per kilogram of the diet: Se PPM 0.3, Zn 120 PPM, Vit A 2.49 TIU, Vit D3 0.45 TIU, Vit E 13.6 TIU/kg, Biotin 0.1 mg , Niacin 10.2 mg, Choline T 223 mg, Vit K 1.3 mg, Thiamin 0.2 mg, Pantothenic acid 20mg, Iron 257 PPM, Copper 10 PPM, Mn 47.17 PPM

Table 2.2 Proximate Analysis Composition of Phase 1 Diets

	Phase 1			
	0% CFP	4% CFP	8% CFP	12% CFP
Taurine	0.24	0.24	0.24	0.25
Hydroxyproline	0.02	0.03	0.03	0.03
Aspartic Acid	2.14	2.23	2.11	1.94
Threonine	0.97	1.00	1.05	1.15
Serine	0.91	0.96	0.95	0.93
Glutamic Acid	3.95	4.08	3.97	3.87
Proline	1.29	1.29	1.32	1.35
Lanthionine	0	0	0	0
Glycine	0.89	0.92	0.89	0.85
Alanine	1.09	1.11	1.17	1.21
Cysteine	0.40	0.42	0.41	0.42
Valine	1.07	1.12	1.11	1.08
Methionine	0.54	0.54	0.60	0.57
Isoleucine	0.97	1.02	0.99	0.95
Leucine	1.83	1.88	1.95	2.02
Tyrosine	0.69	0.72	0.73	0.72
Phenylalanine	1.04	1.08	1.06	1.05
Hydroxylysine	0.02	0.02	0.02	0.02
Ornithine	0.02	0.02	0.02	0.02
Lysine	1.49	1.64	1.66	1.60
Histidine	0.54	0.56	0.54	0.53
Arginine	1.30	1.33	1.24	1.15
Tryptophan	0.24	0.25	0.23	0.23
Total	21.65	22.46	22.29	21.94
Crude Protein	21.39	22.7	22.19	23.52
Moisture	9.12	8.21	8.46	8.48
Crude Fat	3.78	4.54	4.20	4.15
Crude Fiber	2.04	2.02	2.01	2.67
Ash	5.83	6.55	6.49	6.48

Table 2.3 Metabolizable Energy and Amino Acid targets for Phase 1 Diets

	0% CFP	4% CFP	8% CFP	12% CFP
ME. Kcal/kg	3400	3400	3400	3400
Lysine	1.45	1.45	1.45	1.45
SAA:Lys	0.60	0.60	0.60	0.60
Trp:Lys	0.19	0.19	0.19	0.19
Thr:Lys	0.62	0.62	0.62	0.62
Ile:Lys	0.55	0.55	0.55	0.55
Val:Lys	0.67	0.67	0.67	0.67

Table 2.4 Dietary Phase 2 and 3 ingredient composition (kg).

Phase 2					**Phase 3**
	CFP 0%	**CFP 4%**	**CFP 8%**	**CFP 12%**	**Common Diet**
Corn	413	412	411	409	640
Soybean Meal	220	220	220	220	315
Corn Fermented Protein	0	20	40	60	-
Soy Protein Concentrate	60	40	20	0	-
Steam rolled Oats	100	100	100	100	-
Dried Whey	150	150	150	150	-
Soy Oil	20	20	20	20	2
Phosphate 21%	12	12	12	12	9.07
L Lysine 98%	4.5	5.2	5.9	6.6	5.2
DL Methionine	2.4	2.6	2.8	3.0	2.5
Monocalcium Phosphate	-	-	-	-	8.8
Limestone	11	11	11	11	11.3
NaCl	5	5	5	5	5
Vitamin-Mineral Mix	1.8	1.8	1.8	1.8	2.5

[1]Provided per kilogram of the diet: Se PPM 0.3, Zn 120 PPM, Vit A 2.49 TIU, Vit D3 0.45 TIU, Vit E 13.6 TIU/kg, Biotin 0.1 mg , Niacin 10.2 mg, Choline T 223 mg, Vit K 1.3 mg, Thiamin 0.2 mg, Pantothenic acid 20mg, Iron 257 PPM, Copper 10 PPM, Mn 47.17 PPM

Table 2.5 Proximate Analysis Composition of Phase 2 and 3

	Phase 2				Phase 3
	0% CFP	2% CFP	4% CFP	6% CFP	Common Phase
Taurine	0.25	0.23	0.25	0.24	0.25
Hydroxyproline	0.04	0.02	0.03	0.02	0.04
Aspartic Acid	2.00	2.08	2.17	1.86	1.73
Threonine	0.91	0.95	1.06	0.94	0.89
Serine	0.86	0.91	0.96	0.87	0.81
Glutamic Acid	3.63	3.74	3.93	3.45	3.21
Proline	1.14	1.21	1.28	1.19	1.16
Lanthionine	0	0	0	0	0
Glycine	0.78	0.83	0.87	0.79	0.75
Alanine	0.98	1.03	1.09	1.04	0.95
Cysteine	0.32	0.34	0.38	0.35	0.31
Valine	0.94	1.00	1.04	0.93	0.83
Methionine	0.45	0.48	0.5	0.49	0.51
Isoleucine	0.89	0.92	0.96	0.83	0.74
Leucine	1.67	1.74	1.85	1.71	1.57
Tyrosine	0.64	0.67	0.73	0.65	0.61
Phenylalanine	0.96	1.00	1.05	0.93	0.88
Hydroxylysine	0.02	0.02	0.02	0.02	0.02
Ornithine	0.01	0.02	0.02	0.02	0.02
Lysine	1.61	1.57	1.72	1.67	1.43
Histidine	0.49	0.52	0.54	0.49	0.47
Arginine	1.17	1.24	1.30	1.13	1.09
Tryptophan	0.24	0.24	0.22	0.23	0.20
Total	20	20.76	21.97	19.85	18.47
Crude Protein	21.69	21.26	21	21.59	19.3
Moisture	9.95	10.09	9.87	10.03	12.98
Crude Fat	3.93	3.8	4.21	4.18	2.47
Crude Fiber	1.87	1.85	1.74	1.86	2.28
Ash	5.73	5.99	6.03	6.33	5.00

Table 2.6 Metabolizable Energy and Amino Acid targets for Phase 2 and 3

	Phase 2				Phase 3
	0% CFP	4% CFP	8% CFP	12% CFP	Common Diet
ME, kcal/kg	3400	3400	3400	3400	3350
Lysine	1.40	1.40	1.40	1.40	1.35
SAA:Lys	0.60	0.60	0.60	0.60	0.60
Trp:Lys	0.19	0.19	0.19	0.19	0.19
Thr:Lys	0.62	0.62	0.62	0.62	0.62
Ile:Lys	0.55	0.55	0.55	0.55	0.55
Val:Lys	0.67	0.67	0.67	0.67	0.67

Table 2.7 Body weight (kg) of nursery pigs fed diets with graded inclusions of corn fermented protein to replace soy protein concentrate (0%, 4%, 8%, and 12% CFP in Phase 1) and (0%, 2%, 4%, 6% CFP in Phase 2).

	0%	4%	8%	12%	SEM	*P*-value
BW, kg						
Day 0	6.0	6.0	6.0	6.1	0.1	0.99
Day 7	6.6	6.5	6.5	6.4	0.2	0.92
Day 14	8.1	8.0	8.0	7.7	0.3	0.57
Day 21	10.0	9.7	10.1	9.5	0.3	0.43
Day 28	12.7	12.2	12.6	11.7	0.4	0.32
Day 35	16.4	15.7	16.2	15.1	0.5	0.27
Day 42	20.4	19.7	20.3	19.3	0.6	0.47

Table 2.8 Average daily gain (ADG) of nursery pigs fed diets with graded inclusions of corn fermented protein to replace soy protein concentrate (0%, 4%, 8%, and 12% CFP in Phase 1) and (0%, 2%, 4%, 6% CFP in Phase 2) by week and by diet phase.

	0%	**4%**	**8%**	**12%**	**SEM**	***P*-value**
ADG, g/d						
Day 0 to 7	81.67[a]	76.63[a]	84.94[a]	39.96[b]	6.00	0.02
Day 7 to 14	226.10[x]	241.11[x]	211.81[y]	203.03[y]	5.75	0.09
Phase 1 (0 to 14)	153.82[a]	158.87[a]	148.37[a]	121.50[b]	3.26	<0.01
Day 14 to 21	277.30	244.40	284.45	272.23	8.25	0.25
Day 21 to 28	386.68	373.14	360.18	338.47	7.73	0.15
Phase 2 (14 to 28)	329.57	305.32	321.28	300.91	5.59	0.26
Day 28 to 35	527.10	539.86	517.62	503.27	7.74	0.40
Day 35 to 42	593.94	592.55	587.85	596.15	10.43	0.99
Phase 3 (28 to 42)	560.51	566.21	552.73	549.71	7.09	0.85
Day 0 to 42	575.10	569.50	567.90	551.20	4.11	0.25

*a-b within a row, means lacking a common superscript differ ($P<0.05$)

*xy Within a row, means lacking a common superscript are tendencies ($0.10 \leq P \geq 0.05$)

Table 2.9 Average daily feed intake (ADFI) of nursery pigs fed diets with graded inclusions of corn fermented protein to replace soy protein concentrate (0%, 4%, 8%, and 12% CFP in Phase 1) and (0%, 2%, 4%, 6% CFP in Phase 2) by week and by diet phase.

	0%	4%	8%	12%	SEM	*P*-value
ADFI, g/d						
Day 0 to 7	67.41^{x}	72.64^{x}	58.47^{y}	54.17^{y}	2.74	0.06
Day 7 to 14	298.22^{b}	326.83^{a}	316.02^{a}	284.10^{c}	4.70	<0.01
Phase 1 (0 to 14)	182.81^{b}	199.74^{a}	187.25^{ab}	169.12^{c}	3.14	<0.01
Day 14 to 21	322.01^{b}	245.91^{c}	324.47^{b}	415.63^{a}	13.37	<0.01
Day 21 to 28	603.86^{b}	644.89^{a}	569.33^{b}	438.59^{c}	18.01	<0.01
Phase 2 (14 to 28)	462.94	445.40	446.90	427.11	6.96	0.35
Day 28 to 35	805.90	802.10	824.27	775.81	18.43	0.84
Day 35 to 42	1006.89	947.42	918.60	961.38	27.40	0.73
Phase 3 (28 to 42)	906.40	874.75	871.44	868.60	15.27	0.93
Day 0 to 42	426.51^{a}	411.21^{a}	397.40^{b}	368.20^{c}	6.64	0.03

*ab within a row, means lacking a common superscript differ ($P<0.05$)

*xy Within a row, means lacking a common superscript are tendencies ($0.10 \leq P \geq 0.05$)

Table 2.10 Gain-to-feed ratio (G:F) of nursery pigs fed diets with graded inclusions of corn fermented protein to replace soy protein concentrate (0%, 4%, 8%, and 12% CFP in Phase 1) and (0%, 2%, 4%, and 6% CFP in Phase 2) by diet phase.

	0%	**4%**	**8%**	**12%**	**SEM**	***P*-value**
G:F						
Phase 1 (0 to 14)	0.83^{a}	0.72^{bc}	0.76^{ab}	0.68^{c}	0.01	<0.01
Phase 2 (14 to 28)	0.74^{a}	0.69^{bc}	0.74^{ab}	0.69^{c}	0.01	0.03
Phase 3 (28 to 42)	0.63	0.66	0.70	0.66	0.01	0.92
Day 0 to 42	0.74	0.74	0.70	0.68	0.01	0.84

*ab within a row, means lacking a common superscript differ ($P<0.05$)

*xy Within a row, means lacking a common superscript are tendencies ($0.10 \leq P \geq 0.05$)

Table 2.11 Differential sugar absorption test (DSAT) results from pigs fed diets with graded inclusions of corn fermented protein to replace soy protein concentrate (0% and 12% CFP)

	0% CFP	12% CFP	SEM	*P*-value
Lactulose, nM	0.02	0.02	0.00	0.50
Mannitol, nM	0.06	0.05	0.02	0.25
Lactulose: Mannitol	0.34	0.34	0.05	0.98

Figure 2.1 Product information sheet for soy protein concentrate

Arcon® AFF 065-307

Arcon® AFF is soy protein concentrate for use in animal feed applications.

Proximate Data:

% Crude Protein, mfb	65.0 Minimum
% Moisture (%)	9.00 Maximum
% Ash	7.00 Maximum
% Crude Fat	0.30 Minimum
% Crude Fiber	5.00 Maximum

Typical Amino Acids (g/100 g protein)	
Aspartic Acid	10.8
Threonine*	3.7
Serine	4.5
Glutamic Acid	17.1
Proline	5.0
Glycine	4.1
Alanine	4.1
Cystine	1.3
Valine*	4.9
Methionine*	1.3
Isoleucine*	4.7
Leucine*	7.5
Tyrosine	3.4
Phenylalanine*	4.8
Histidine*	2.5
Lysine*	6.1
Arginine	6.9
Tryptophan*	1.2

*Essential Amino Acids

Typical Minerals (mg/100g)	
Potassium	2200-2800
Calcium	200-400
Phosphorus	800-1200
Iron	8-13
Magnesium	200-400

PHYSICAL PROPERTIES
Bland flavor, 100 mesh powder

PACKAGING
Available in 20 kg. net weight, multi-wall paper bags, 1800 lb. and 2000 lb. super sacks.

STORAGE
Storage below 75° F (23.9° C) and 60% relative humidity promotes longer shelf life.
Recommended shelf life, as packed, is three years.

LABELING: INGREDIENT STATEMENT
Soy Protein Concentrate.

Archer Daniels Midland Company
4666 East Faries Parkway • Decatur, Illinois 62526 • 217-424-5200 (phone)
www.adm.com

EVALUATING THE INCLUSION OF A *LACTOBACILLUS*-BASED PROBIOTIC OR A *BIFIDOBACTERIA*-BASED POSTBIOTIC IN THE NURSERY PERIOD ON GUT HEALTH, MICROBIAL POPULATIONS, AND PIG PERFORMANCE THROUGH TO MARKET

3.1. ABSTRACT

Probiotics and postbiotics may be beneficial alternatives to reduce antibiotic use in swine production. Due to the numerous environmental and biological stressors that may affect the pig's health entering a new barn, a beneficial gut microbiota that can regulate and reinforce the immune system by altering the digestive tract environment, regulating pH, and competing with hostile bacteria that could harm the animal should be beneficial. Probiotic and postbiotic feed additives can also reduce mortality and the use of antibiotic medicine, by regulating the balance of the intestinal flora, competing with intestinal pathogenic bacteria, and enhancing immunity. Therefore, this study aimed to evaluate the inclusion of a *Lactobacillus*-based probiotic or a *Bifidobacteria*-based postbiotic in nursery pig diets on gut health, microbial populations, and carcass value of finished pigs. The trial involved 1,040 pigs allocated to 40 pens of 26 pigs per pen, with a starting body weight (BW) of 6.1 ± 0.1 kg. Pens were assigned to one of 4 dietary treatments fed over four dietary phases: 1) Control, 2) Control + 0.1% inclusion of *Lactobacillus*-based probiotic (0.1% LacPro), 3) Control + 0.2% inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or 4) Control + 0.2% inclusion of *Bifidobacteria*-based postbiotic (BifPos). Pens of pigs were weighed at barn entry, day 10, day 21, day 47, day 70, day 105, and day 135. Feed remaining on each weigh day was calculated according to a prepared calibration curve accounting for the distance from the top of the feeder to the top of the feed and the density of the feed. Fecal samples were collected on day 10 and day 47 to evaluate microbial populations. To measure gut health parameters, on day 10, 40 pigs were euthanized,

providing 10 jejunal and ileal tissue samples per treatment that were then measured for villus height (VH), crypt depth (CD), and villus height to crypt depth ratio (VCR). On day 10, pigs provided with the Control diet had lower ($P = 0.05$) average daily feed intake (ADFI) than pigs fed with diets containing the probiotics and postbiotic (150 vs 177 ± 3.38 g/d). Histological analysis from day 10 indicated a greater ($P < 0.02$) VCR in the ileal tissue from pigs fed 0.1% LacPro, 0.2% LacPro, and 0.2% BifPro compared to Control (1.04, 1.21, and 1.18 µm vs. 0.99 µm). An increased abundance ($P < 0.03$) of *Lactobacillaceae* family in feces from 0.2% LacPro and 0.2% BifPos compared to Control (10.38% and 10.78% vs. 3.53%) on day 10 was observed. The increased GIT surface area and greater abundance of *Lactobacillaceae* in both LacPro and BifPos-fed pigs may provide a more LAB microbiota capable of benefiting the host with less pathogen exposure.

Keywords: Probiotics, nursery diets, gut morphology, microbiome

3.2. INTRODUCTION

Probiotics, also known as direct-fed microbial (DFM), include lactic acid bacteria (LAB) strains such as *Lactobacillus*, *Bacillus*, and *Enterococcus* and are among the most used probiotics for pigs (Sato et al., 2019). Probiotics in the swine industry may have numerous benefits for the pig, including modifying the gastrointestinal tract (GIT) microbiota population and creating a balanced microbiota to improve gut health. A well-balanced microbiome can lead to disease resistance, reduce shedding of pathogens and disease symptoms, and improve health status (Upadhaya et al., 2015). Probiotics may be used to reduce the use of antibiotic growth promoters (AGP). Antibiotic growth promoters are used to advance growth and enhance feed efficiency.

The EU has banned any imported meat products raised with AGP since 2022 (Rahman et al., 2022).

The implementation of new guidelines by the U.S. Food and Drug Administration (FDA) in 2023 prohibited the sale of over-the-counter medically important antimicrobial drugs aiming to combat the escalating issue of bacterial resistance to antibiotics (Center for Veterinary Medicine, 2023). Antibiotic growth promoters contribute to the rise of multidrug-resistant pathogenic bacteria (Rahman et al., 2022).

Probiotics may be especially helpful for weaned pigs, as they still have an underdeveloped GIT at this production stage, and diarrhea incidences are often high. Probiotics can reduce the proliferation of pathogenic microorganisms by establishing beneficial bacteria, contributing to the defense against pathogenic microbial invasion by lowering the pH in the GIT and enhancing gut barrier function by providing energy to intestinal epithelial cells (Su et al., 2022). Weaning induces morphological, enzymatic, and inflammatory changes in the GIT, leading to a breakdown in intestinal barrier function. Studies have shown decreased electrical resistance and increased permeability in weaned pigs, accompanied by an upregulation of proinflammatory cytokines and activation of intestinal mast cells. Additionally, weaning age significantly impacts intestinal barrier function, with younger pigs exhibiting more severe injuries. Long-term effects of weaning include lasting changes in immune responses and enteric nervous system function. These findings suggest that weaning-induced alterations in the GIT barrier and nervous system may increase disease susceptibility in early-weaned pigs (Moeser et al., 2017).

The FAO/WHO defines probiotics as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.” These live

microorganisms improve the intestinal microbial balance (Bajagai et al., 2016). There are many benefits to including probiotics in swine diets, such as reducing the need for antibiotics, early establishment of a healthy gut microbiome, reduction of diarrhea incidences, disease resistance, improved feed intake, and gut integrity (Liao & Nyachoti, 2017). Good GIT integrity includes well-formed villi and crypts in the intestinal wall. Villi are finger-like projections that form from the mucosal surface which increase the surface area available for absorption of nutrients. Villi contain a network of blood vessels and lymphatic vessels that transport absorbed nutrients to the bloodstream. Crypts are invaginations located between the villi that contain stem cells that continuously divide and differentiate into various cell types including enterocytes (absorptive cells), goblet cells, paneth cells (antimicrobial peptides), and enteroendocrine cells (hormone secretion involved in digestion and appetite regulation). These crypts play a vital role in replenishing the epithelial cells that line the villi, ensuring the integrity and functionality of the intestinal barrier (Bowen, 2019).

Postbiotics are metabolic products derived from probiotic bacteria grown outside of the host that exert beneficial effects on the host directly or indirectly (Tsilingiri, 2013). Some of the products derived from probiotics are lactic acids, bacteriocins, short-chain fatty acids (SCFAs), exopolysaccharides (extracellular carbohydrate polymers), cellular wall fragments, and bacterial deoxyribonucleic acid (DNA), which have antimicrobial, immunomodulatory, and anti-inflammatory properties (Zhong et al., 2022). The advantage of postbiotics over probiotics is that probiotics may have some potential risks, such as genetic instability, rate of survivability, or in situ toxin production, and these issues do not occur in postbiotics because they are inanimate microorganisms plus end products (Zhong et al., 2022).

The health and efficiency of the GIT may depend on the presence of specific species and the diversity of the GIT microbiome. Some beneficial GIT bacteria include *Lactobacillus*, *Bifidobacterium*, and various species of *Firmicutes* and *Bacteroidetes*. A lack of diversity in the microbiome can cause dysbiosis (Lozupone et al., 2012). Dysbiosis is an imbalance in bacterial composition, changes in bacterial metabolic activities, or changes in bacterial distribution within the gut. These effects have been associated with some diseases and GIT conditions (Lozupone et al., 2012). Lactic acid bacteria, mentioned above, help in the breakdown of complex carbohydrates, synthesis of certain vitamins, and maintenance of intestinal health. Especially for a pig at a young age, a well-structured GIT that prevents pathogen entry into the body is of major importance. A healthy GIT can help the weaned pig grow and develop without any setbacks from pathogens. One of the parameters to evaluate or define a well-structured GIT is the VCR. This is a common method used to assess the health and functionality of the intestinal mucosa and nutrient absorption efficiency. It is an effective parameter for assessing intestinal integrity; when this ratio increases, it is assumed that digestion and absorption are improved (Wilson et al., 2018). Studies in poultry suggest that microbial synthesis of fermented products such as SCFAs modulates intestinal epithelium proliferation and exposure to LAB has been reported to accelerate the crypt-villus axis of intestinal enterocytes by activating integrin collagen receptors (Šefcová et al., 2023). Providing probiotics and postbiotics to nursery pig diets can aid in developing a healthier GIT which can then positively impact survival through the many stressors it may encounter.

This trial aimed to evaluate the effects of including two levels of a *Lactobacillus*-based probiotic or one level of a *Bifidobacteria*-based postbiotic in the nursery period

on pig performance, gut morphology, microbial populations, and hot carcass weight of the finished pigs.

3.3. MATERIALS AND METHODS

3.3.1. Animals, Housing and Feeding

A total of 1,040 pigs (initial body weight (BW) 6.1 ± 0.1 kg) were selected for this specific study from a population of 1,244 pigs (PIC 800 x PIC) delivered to the South Dakota State University Off-site commercial research facility. Pigs were allocated to 40 pens, 20 in each room of the facility, blocked by weight and barn location to have 26 pigs per pen (13 barrows and 13 gilts/pen; n=10 pens/treatment). Each pen provided an area of 3.1 m x 6.9 m (approximately 0.82 m^2 per pig). Pens were assigned to one of 4 dietary treatments: 1) Control, 2) Control + 0.1% inclusion of Lactobacillus-based probiotic (0.1% LacPro), 3) Control + 0.2% inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), 4) Control + 0.2% inclusion of *Bifidobateria*-based postbiotic (BifPos). Feeding order of the diets was established to reduce any potential dietary treatment carry-over: Control, 0.1% LacPro, 0.2% LacPro, Control, then finally 0.2% BifPos; Control started the next feeding cycle. The number of feeding cycles per day were adjusted depending on the feed intake of the barn. Pens were started with two cycles per day (i.e. every twelve hours), followed by four cycles per day (i.e. every six hours), and finished with six feeding cycles per day (i.e. every four hours). Diets were fed in 2, 2, and 6 phases in the nursery, grower, and finish periods, respectively. Pigs in this study were provided a total of 8.8 kg/pig feed budget for the nursery phases: 4.3 kg/pig for dietary Phase 2 and 4.5 kg/pig for dietary Phase 3. Feed budget for the grower and finishing dietary phases were established as follows: Phase 4: 24 kg/pig; Phase 5: 19 kg/pig; Phase 6: 43 kg/pig; Phase 7: 49 kg/pig; Phase 8: 50kg/pig; Phase 9: 48 kg/pig; and Phase 10: 21 kg/pig. The

probiotics and postbiotic were supplemented until the start of Phase 4 of feeding due to a limitation of product availability (Tables 3.1 & 3.2). Diet formulations provided lysine and energy that met or exceeded NRC (2012) recommendations for each diet.

3.3.2. Experimental Design

The experimental design used for this study was a complete block design with 10 blocks per treatment (five per room) and every treatment was represented in every section block of the barn. One room of the barn was also used for a completely separate behavioral study during the nursery phase only.

3.3.3. Feed Measurements and Calculations

All pens contained one five-hole dry feeder and two cup waterers for *ad libitum* access to feed and water, respectively. The facility was equipped with a Feedlogic M-Series (Feedlogic ComDel Innovation, Wilmar, MN) system for feeding. Feed remaining on weigh day was calculated according to a prepared calibration (Growth performance calculations; page 47) curve by measuring the distance from the top of the feeder to the top of the leveled feed and its density, as previously described (Clizer et al, 2022). The feed disappearance was calculated using the drop history recorded by the Feedlogic program providing the total feed delivered from the previous weigh day to the current weigh day and subtracting the feed remaining on the same day the experimental pen was weighed.

3.3.4. Pen Weights and Growth Performance Calculations

Pens of pigs were weighed at barn entry and Day 10 and Day 21 because these day marked the end of first and second nursery diets (dietary Phases 2 and 3). Thereafter, pigs were weighed approximately every month starting at Day 47 then

continuing on Day 70, Day 105, and Day 135. Growth performance parameters, BW, average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were calculated by a series of formulas related to pig day. Pig day includes the removal date(s) and weight(s) of any pig(s) between weigh day without compromising the calculation of performance results. Specifically, pig day accounts for the day from the last weigh day to the current, multiplied by the number of pigs in that particular pen on the weigh day, and lastly, adding the day(s) any removed pigs were on trial and the weight(s) of the pig(s) on the day of removal.

Growth performance calculations:

$$ADG = (current\ pen\ weight - previous\ pen\ weight + removed\ pig\ weights) \div pig\ days$$

$$ADFI = (feed\ delivery - feed\ WB) \div pig\ days$$

$$G:F = ADG \div ADFI$$

3.3.5. Marketing of Finished Pigs

Pigs were marketed over four weeks by selecting the heaviest four pigs per pen the day before. Pigs were visually selected by the biggest body size from the average of the pen at each marketing event. Each truckload had, on average, 160 pigs from the experimental pens. There were two trucks per week, except the last week, which was only one truck, for a total of seven trucks.

3.3.6. Villus Height and Crypt Depth Measurements

To measure gut integrity, intestinal VH and CD were evaluated. On day 10 after weaning, one pig per pen, which appeared healthy and had a good average weight among all the pigs in the pen, was selected randomly and euthanized by a non-penetrating captive bolt gun (n=40; 10 per treatment). After being euthanized, the pig was transported to a sterilized table, where an incision was made across the belly in a caudal direction and the intestinal tract laid onto the table where jejunal tissue (5cm at the midpoint of the small intestine) and ileal tissue (5cm section at a point 10 cm proximal from the ileocecal junction) were rinsed with a solution of distilled water and placed in a bottle that contained 15 ml paraformaldehyde for conservation for later processing for histological analysis. For the histological analysis, samples were placed on slides and colored with Methylene blue dye. The distance of the VH and the CD from both the ileum and jejunum samples was measured using an Olympus CK2 microscope. The average of the ten measurements from each sample was used to calculate an overall set of measurements for each pen.

3.3.7. Fecal Sample Collection

Fecal sample collections by rectal palpitation were done on Day 10, Day 21, and Day 47. Samples were then put in a 5 ml conical tube put on ice, and later stored frozen at -20°C until they were processed for microbic genome extraction.

3.3.8. Fecal Sample Processing

Fecal samples were thawed at room temperature, and a small portion, previously stirred to ensure a homogeneous sample, then placed in 2.5 ml tubes and a protocol of Isolation of microbial genomic DNA and sequencing of 16S rRNA gene amplicons

was performed for the Control treatment, 0.2% *Lactobacillus*-based probiotic and 0.2% *Bifidobacteria*-based postbiotic for the time points of Day 10 and Day 47. Only three treatments during the supplementation period were measured due to anticipated limited difference between the 0.1% and 0.2% LacPro treatments. A total of sixty samples were processed as described below, thirty at each time point.

3.3.9. Isolation of Microbial Genomic DNA and Sequencing of 16s rRNA Gene Amplicons

Microbial genomic DNA was extracted from individual samples using a bead-beating plus column approach (Yu, 2004), including the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The V1-V3 regions of the bacterial 16S rRNA gene were targeted by PCR using the universal forward 27F-5'AGAGTTTGATCMTGCTCAG and reverse 519R 5'GWATTACCGCGCGCGCTG primers (Lane, 1985). Purified microbial genomic DNA samples were submitted to Molecular Research DNA (MRDNA, Shallowater, TX, USA) for V1-V3 amplification and amplicon sequencing with the Illumina MiSeq 2 x 300 platform to generate overlapping paired-end reads.

3.3.10. Bacterial Composition Analyses

Custom-written Perl scripts and publicly available software were employed to process sequence data. Initial screening of sequences from merged overlapping paired-end reads, corresponding to V1-V3 amplicons of the 16S rRNA bacterial gene, involved ensuring the presence of intact 27F and 519R primer sequences, a minimal average Phred quality score of Q33, and a length between 400 and 580 nucleotides (Poudel et al., 2022). After quality filtering, amplicon sequences underwent alignment and clustering into operational taxonomic units (OTUs) using a sequence dissimilarity cutoff of 4%, a threshold deemed more suitable for the V1-V3 region compared to the

commonly used 3% cutoff for clustering 16S rRNA sequence data (Kim et al., 2011; Johnson et al., 2019)

Following OTU clustering, three independent approaches were employed to identify artifacts (Poudel et al., 2022). Firstly, the 'chimera.slayer' (Haas et al., 2011) and 'chimera. chime' (Edgar et al., 2011) commands from the MOTHUR (v.1.36.1) open-source software packages (Schloss et al., 2009) were utilized to screen for chimeric sequences. Secondly, an alignment search-based approach assessed the 50 and 30 ends of OTUs, designating those with more than 5 nucleotides missing from either end as artifacts based on comparison to their closest match in the NCBI 'nt' database using BLAST (Altschul et al., 1997).

Thirdly, OTUs with only one or two assigned reads underwent additional screening, retaining only sequences with a perfect or near-perfect match (maximum 1% dissimilar nucleotides) to a sequence in the NCBI 'nt' database. Subsequently, flagged OTUs and their assigned reads were removed from further analyses. The curated OTUs were then subjected to taxonomic assignment using two strategies. Phylum and family-level affiliations for all OTUs were determined using the RDP Classifier, which is a software tool that assigns rRNA sequence data (Wang et al., 2007), while the most abundant OTUs had their closest valid relatives identified through BLAST searches against the 'refseq_rna' database (Altschul et al., 1997). Alpha diversity indices, including 'Observed OTUs,' 'Chao,' 'Ace,' and 'Shannon,' were determined using the 'summary.single' command in the MOTHUR (v.1.44.1) software package (Schloss et al., 2009). For beta diversity analysis, Bray–Curtis distances were initially calculated with 'summary.shared,' followed by Principal Coordinate Analysis (PCoA) using the 'pcoa' command in MOTHUR (v.1.44.1).

3.4. STATISTICAL ANALYSIS

3.4.1. Growth Performance

The BW, ADG, ADFI, G:F and carcass data were analyzed using a generalized linear mixed model using the PROC GLIMMIX procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC), considering the main effect of dietary treatment where the pen was the experimental unit. An LSMEANS statement was included for the fixed effects, which represent the mean response for each level of a factor adjusted for any other variables. Pens in the behavioral study were considered an effect for the nursery phases, which was considered a variable in the statistical analysis. Any statistical difference was considered with a *P*-value ≤0.05. A trend was considered with a *P*-value of <0.10 and >0.05.

3.4.2. Mortality Data

To analyze mortality percentages, the PROC FREQ procedure of SAS (Version 9.4, SAS inst. Inc., Cary, NC) was used to perform a Chi-squared test to evaluate categorical values that represent every removal percentage per treatment. Any statistical difference was considered with a *P*-value of ≤0.05.

3.4.3. Histology Data

Histological data was analyzed using the PROC GLIMMIX procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC), where dietary treatment was considered the main effect. Any statistical difference was considered with a *P*-value ≤0.05.

3.4.4. Microbiome Data

For the statistical analysis of taxonomic groups and most abundant OTUs (non-parametric data), the Wilcoxon rank-sum test was performed in 'R' (Version 3.6.0). A threshold of $P \leq 0.05$ was considered significant, and $0.05 > P < 0.10$ is considered a trend.

3.5. RESULTS

The dietary inclusion of 0.1% or 0.2% *Lactobacillus*-based probiotic or 0.2% *Bifidobacteria*-based postbiotic to the Control diet during the nursery phase had no impact ($P \geq 0.64$) on the average BW of the pigs throughout the study period (Table 3.4). Consistent with the BW results, there were no differences ($P \geq 0.78$) in ADG between pigs fed diets without or with additions of 0.1% or 0.2% of a *Lactobacillus*-based probiotic or 0.2% a *Bifidobacteria*-based postbiotic during the nursery phase (Table 3.5). Pigs fed diets with 0.1% or 0.2% dietary additions of a *Lactobacillus*-based probiotic or 0.2% *Bifidobacteria*-based postbiotic had significantly greater ADFI ($P = 0.05$) compared to pigs fed the Control diet during Day 0 to 10 (Table 3.6). However, as the study progressed to Day 10 to 21, 21 to 47, 47 to 70, 70 to 105, and 105 to 135, no other differences ($P \geq 0.36$) in ADFI between the groups were measured. The dietary 0.1% and 0.2% inclusion of a *Lactobacillus*-based probiotic or 0.2% of a *Bifidobacteria*-based postbiotic during the nursery period had no significant impact ($P \geq 0.28$) on G:F of newly weaned, growing, and finishing pigs (Table 3.7).

Overall, the inclusion of 0.1% and 0.2% inclusion of a *Lactobacillus*-based probiotic or 0.2% of a *Bifidobacteria*-based postbiotic did not influence ($P \geq 0.08$) hot carcass weight throughout every marketing event (Table 3.8).

The mortality of pigs fed diets with no dietary inclusion of probiotic or postbiotic, 0.1% dietary inclusion of Lactobacillus-based probiotic, 0.2% dietary inclusion of *Lactobacillus*-based probiotic, or 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (Table 3.9) had no statistical difference amongst treatments ($P = 0.69$).

Histological analysis of ileal tissue from day 10 (Table 3.10) indicated that inclusion of 0.2% *Lactobacillus*-based probiotic and 0.2% *Bifidobacteria*-based postbiotic increased ($P = 0.02$) VCR compared to pigs fed Control plus 0.1% *Lactobacillus*-based probiotic and Control. Furthermore, pigs fed Control plus 0.1% of *Lactobacillus*-based probiotic had greater VCR compared to pigs fed Control.

Results from the histological analysis demonstrated that there were no differences ($P > 0.15$) in VH, CD, and VCR in jejunal tissues from Day 10 between pigs fed Control, 0.1% LacPro, 0.2% LacPro, and BifPos (Table 3.11).

On day 10, microbial populations of pigs fed 0.2% *Lactobacillus*-based probiotic and 0.2% *Bifidobacteria*-based postbiotic had an increased percentage ($P = 0.03$) of the species *Lactobacillaceae* compared to pigs fed Control (Table 3.12). Microbial populations of pigs fed a 0.2% *Lactobacillus*-based probiotic or a 0.2% *Bifidobacteria*-based postbiotic tended ($P = 0.08$) to have an increased percentage of *Lactobacillaceae* species compared to pigs fed a Control diet on day 47 (Table 3.13).

Principal Coordinate Analysis (PCoA) performed using a Bray-Curtis distance matrix from feces collected on Day 10 and Day 47 from pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control

plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos) show that the microbial populations from Day 10 statistically differ from those on Day 47 with minimal differences between treatment groups at a given day (Figure 3.1).

3.6. DISCUSSION

3.6.1. Growth Performance

Results from the trial determined that pigs fed 0.1% or 0.2% *Lactobacillus*-based probiotic or 0.2% *Bifidobacteria*-based postbiotic had greater ADFI from Day 0 to Day 10 compared to pigs fed the Control diet. In accordance with our outcome, Yang et al. (2020) reported that piglets fed *L. plantarum* at a concentration of 0.03% and 0.06% showed improved ADFI from weeks 1 to 3 compared to the basal diet group. However, Based on our findings, the inclusion of *Lactobacillus*-based probiotics and *Bifidobacteria*-based postbiotics does not improve the piglet's weight gain and, therefore, there is no dietary impact.

Literature regarding the use of probiotics is inconsistent with respect to an effect on growth performance. Many variations have been published where the inclusion of probiotics may decrease, have no difference, or may increase ADG, feed conversion ratio, or ADFI. Lv et al. (2015) reported a negative impact on ADFI in weaned pigs when including Lactobacillus acidophilus; however, pigs fed that probiotic had significantly increased ADG. Kantas et al., (2015) results showed an improvement in feed intake with inclusion of a probiotic containing *B. toyonensis* in weaned pigs.

3.6.1 Intestinal Tissue Villus Height and Crypt Depth

Wang et al. (2022) studied the developmental changes in the intestinal epithelium in weaning pigs (Landrace x Yorkshire x Duroc; 6.55 kg), where they sampled 2cm of

the duodenum, jejunum, and ileum of 8 piglets at different time points after weaning (day 0, 1, 3, 7, and 14). On day 14, the average villus height was 343.7 μm for the jejunum and 328.83 μm for the ileum. Their results in crypt depth on day 14 were 195.47 μm and 224.60 μm for the jejunum and ileum, respectively. Compared to our findings the average villi height in the jejunum and ileum are 356.4 μm and 282.1 μm for the ileum and jejunum. Our crypt depth measurements were 258.1 μm and 298.3 μm for the ileum and jejunum. According to the literature, long villi are associated with increased total luminal absorptive area, increased digestive enzyme action, and higher transport of nutrients (Laudadio et al., 2012). Shallow crypts represent the prolonged survival of villi without the need for renewal (Miles et al., 2006). The results from the crypt depth from our study suggest that during that period (day 10), there is a greater cell turnover rate due to the increased depth of the crypt, meaning more cells are being regenerated at the time. This, overall results in lower nutrient absorption (Marchewka et al., 2021).

Zhaxi et al. (2020) noted that nursery pigs fed yeast-based probiotics exhibited the most intact duodenal mucosa, characterized by thick and healthy villi, although some partial villus loss was observed in the treated group. Similarly, Pupa et al. (2021) found that weaned pigs receiving encapsulated *L. plantarum*, alginate, and gelatin displayed enhanced intestinal integrity and longer villi in the jejunum. These findings align with our observations, where pigs fed *Lactobacillus*-based probiotics or *Bifidobacterium*-based postbiotics demonstrated increased VCR in the ileum on day 10. Evaluating the VCR is crucial for assessing improvements in nutrient absorption across the gastrointestinal tract because there is likely more surface area, meaning more absorption capacity.

Tsukahara et al. (2011) administered a postbiotic from *E. faecalis* to twenty-one-day-old pigs. Results showed that after ten days of supplementation, there was a significant reduction in villus atrophy, in other words, damaged villi. This is relevant to our study because it demonstrates that probiotics increase VCR and reduce damage in the intestine, representing a therapeutic action in the GIT.

According to a study by Yang et al. (2015) gene expression of tight junction proteins in newborn piglets was upregulated by the intake of *L. reuteri*, leading to an enhanced intestinal barrier function. Similarly, Yi et al. (2018) revealed a positive outcome in weaned pigs who were supplemented with an isolated strain of *L. reuteri* (5 x 1010 CFU/kg), showing an increase in VCR in both the jejunum and ileum. This was possibly due to *L. reuteri* -induced increase in production of interleukin-22, which is associated with the reduction of intestinal inflammation and in maintaining the integrity of the intestinal barrier and wound healing in the intestine (Yi et al., 2018).

3.6.2. Mortality

Our research found no difference in mortality when feeding pigs with 0.1%, 0.2%, and 0.2% *Lactobacillus*-based probiotic and 0.2% *Bifidobacteria*-based postbiotic inclusion. There are no consistent published data definitively proving a reduction in mortality when including *Lactobacillus* or *Bifidobacteria* probiotics. However, many published articles have found that including probiotics in weaned pig diets reduces PWD. Post-weaning diarrhea is a disease with high morbidity, consequently leading to productivity loss and mortality (Canibe et al., 2022). Suo et al. (2012) findings showed that supplementing weaned pigs with *L. plantarum* ZJ316 in drinking water alleviated PWD more effectively than dietary antibiotics. The authors suggested that

the observed probiotic effects might be related to the growth inhibition of opportunistic pathogens and the promotion of increased villus height along the GIT.

3.6.3. Microbiome

Ten day after the start of the trial, pigs fed with *Lactobacillus*-based probiotics or *Bifidobacterium*-based postbiotics had increased percentage of the *Lactobacillaceae* population compared to pigs fed the Control diet. *Lactobacillus* is part of the *Lactobacillaceae* family, is involved in the digestion of complex carbohydrates not digested by the host in the colon, and also participates in the degradation of lipids and simple sugars in the duodenum and jejunum (Valeriano et al., 2017).

According to our findings from the mean relative abundance percentage at Day 47 (Table 3.10), the treatment effect continued until the end of the supplementation period, where the percentage of *Lactobacillaceae* family in the treatments 0.2% LacPro and BifPos tended to be higher than the Control treatment. Also, the Control treatment tended to have a higher percentage of *Streptococcaceae*. A greater concentration of *Lactobacillaceae* can be generated during microbial fermentation; *Lactobacillus* can use the nutrients in feed to grow and produce organic acids, enzymes, extracellular polysaccharides, and other metabolites.

3.7. CONCLUSION

The inclusion of *Lactobacillus*-based probiotics and *Bifidobacteria*-based postbiotics in weaned pigs increased daily feed intake during the first ten days of the study. However, it did not impact weight gain throughout the trial. These findings suggest that the inclusion of these probiotics and postbiotics is not effective at the inclusion levels of 0.1% and 0.2%. The impact of probiotics and postbiotics may be beneficial to the newly weaned pig helping it to withstand the stressors during this

period, increasing the percentage of beneficial bacteria in the GIT, as we found in our study, with the increase in *Lactobacillaceae*.

Table 3.1 Phase 2 and 3 Ingredient Composition of Control, 0.1% *Lactobacillus*-based Probiotic, 0.2% *Lactobacillus*-based Probiotic, and 0.2% *Bifidobacterium*-based Postbiotic (kg)

	Phase 2				**Phase 3**			
	Control	0.1% LacPro	0.2% LacPro	0.2% BifPos	Control	0.1% LacPro	0.2% LacPro	0.2% LacPro
Corn	233	232	232	232	477	432.9	432.9	432.9
Soybean Meal					151	151	151	151
DDGS					50	50	50	50
Monocalcium Phosphate 21%	6.4	6.4	6.4	6.4	9.5	9.5	9.5	9.5
Limestone	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7
Corn Oil	6.5	6.5	6.5	6.5	6.3	6.3	6.3	6.3
Lysine HCL	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
Salt	2.5	2.5	2.5	2.5	4.5	4.5	4.5	4.5
Zinc Oxide	3.9	3.9	3.9	3.9	3.0	3.0	3.0	3.0
Threonine PRO 80%	3.2	3.2	3.2	3.2	3.0	3.0	3.0	3.0
DL-Methionine-99%	3.0	3.0	3.0	3.0	2.5	2.5	2.5	2.5
L-Valine	0.2	0.2	0.2	0.2	0.6	0.6	0.6	0.6
L-Tryptophan	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PGF VTM[1]					0.5	0.5	0.5	0.5
Premix*		1.0	1.0	1.0		1.0	1.0	1.0
1200 Crumble[2]	598.4	598.4	598.4	598.4	274.5	274.5	274.5	274.5
Steam Rolled Oats	124	124	124	124				
Total	1000	1000	1000	1000	1000	1000	1000	1000

[1]Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin,
17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe
from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.
[2]Whey permeate, soybean meal, Soy Protein, Porcine Specialty Protein, steamed rolled oats, Fat, Biological Protein, L-Valine, VTM

Table 3.2 Proximate Analysis Composition of Phase 2 Diets

	Phase 2			
	Control	0.1% LacPro	0.2% LacPro	0.2% BifPos
Taurine	0.28	0.28	0.29	0.30
Hydroxyproline	0.02	0.03	0.02	0.02
Aspartic Acid	1.67	1.87	1.53	1.24
Threonine	1.19	0.91	0.86	0.99
Serine	0.77	0.84	0.72	0.60
Glutamic acid	3.02	3.34	2.80	2.36
Proline	0.94	1.01	0.88	0.79
Lanthionine	0.00	0.00	0.02	0.00
Glycine	0.73	0.82	0.68	0.57
Alanine	0.84	0.91	0.79	0.70
Cysteine	0.35	0.36	0.32	0.26
Valine	0.99	1.15	0.93	0.79
Methionine	0.56	0.42	0.46	0.76
Isoleucine	0.86	0.97	0.78	0.64
Leucine	1.44	1.58	1.34	1.18
Tyrosine	0.65	0.71	0.61	0.52
Phenylalanine	0.90	0.98	0.83	0.70
Hydroxylysine	0.06	0.06	0.05	0.05
Ornithine	0.01	0.01	0.01	0.01
Lysine	1.40	1.25	1.26	1.36
Histidine	0.42	0.47	0.39	0.34
Arginine	1.06	1.19	0.98	0.80
Tryptophan	0.24	0.27	0.22	0.24
Total	18.40	19.43	16.77	15.22
Crude Protein	18.30	20.12	17.41	15.46
Moisture	10.13	10.01	10.19	11.04
Crude Fat	4.89	5.53	4.57	3.87
Crude Fiber	1.42	1.21	1.36	1.20
Ash	6.43	6.16	6.40	7.10

*Percentage of grams per 100 grams of sample

Table 3.3 Proximate Analysis Composition of Phase 3 Diets

	Phase 3			
	Control	0.1 % LacPro	0.2% LacPro	0.2% BifPos
Taurine	0.28	0.28	0.28	0.28
Hydroxyproline	0.03	0.04	0.03	0.02
Aspartic Acid	1.71	1.81	1.75	1.77
Threonine	0.98	1.02	1.02	1.03
Serine	0.80	0.83	0.77	0.79
Glutamic acid	3.09	3.17	3.11	3.25
Proline	1.04	1.06	1.05	1.10
Lanthionine	0.00	0.00	0.02	0.00
Glycine	0.73	0.77	0.74	0.76
Alanine	0.90	0.93	0.91	0.96
Cysteine	0.31	0.32	0.32	0.30
Valine	0.92	1.00	1.02	1.01
Methionine	0.60	0.48	0.64	0.53
Isoleucine	0.79	0.85	0.85	0.86
Leucine	1.57	1.60	1.58	1.66
Tyrosine	0.67	0.71	0.68	0.70
Phenylalanine	0.92	0.95	0.93	0.96
Hydroxylysine	0.07	0.07	0.06	0.06
Ornithine	0.01	0.01	0.01	0.01
Lysine	1.40	1.42	1.45	1.40
Histidine	0.45	0.47	0.46	0.48
Arginine	1.06	1.14	1.10	1.11
Tryptophan	0.26	0.26	0.26	0.26
Total	18.59	19.19	19.04	19.30
Crude Protein	19.43	19.98	19.57	20.06
Moisture	11.31	10.66	10.61	10.97
Crude Fat	2.96	3.37	3.27	3.19
Crude Fiber	1.87	1.96	1.91	1.96
Ash	5.84	6.10	6.24	6.09

*Percentage of grams per 100 grams of sample

Table 3.4 Phase 4 Ingredient Composition of Control, 0.1% *Lactobacillus*-based Probiotic, 0.2% *Lactobacillus*-based Probiotic, and 0.2% *Bifidobacterium*-based Postbiotic (kg)

	Phase 4			
	Control	0.1% LacPro	0.2% LacPro	0.2% BifPos
Corn	571	570	570	570
Soybean Meal	272	272	272	272
DDGS	100	100	100	100
Corn Oil	19.4	19.4	19.4	19.4
Limestone	9.2	9.2	9.2	9.2
Monocalcium Phosphate 21%	8.5	8.5	8.5	8.5
Salt	6.0	6.0	6.0	6.0
Lysine HCL	5.7	5.7	5.7	5.7
Threonine PRO 80%	2.6	2.6	2.6	2.6
DL-Methionine-99%	2.0	2.0	2.0	2.0
PGF VTM[1]	1.0	1.0	1.0	1.0
L-Valine	0.5	0.5	0.5	0.5
L-Tryptophan	0.5	0.5	0.5	0.5
Premix*		1.0	1.0	1.0
Total	1000	1000	1000	1000

[1]Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite.

Micronutrients: (Copper Chloride at 0.3 kg)

Table 3.5 Proximate Analysis Composition of Phase 4 Diets

	Phase 4			
	Control	0.1% LacPro	0.2% LacPro	0.2% BifPos
Taurine	0.26	0.27	0.27	0.26
Hydroxyproline	0.02	0.03	0.02	0.03
Aspartic Acid	1.98	1.79	1.95	1.83
Threonine	0.91	0.95	0.96	0.90
Serine	0.89	0.80	0.88	0.85
Glutamic acid	3.56	3.24	3.51	3.35
Proline	1.19	1.15	1.23	1.17
Lanthionine	0.00	0.00	0.00	0.00
Glycine	0.84	0.78	0.82	0.79
Alanine	1.03	0.99	1.05	1.01
Cysteine	0.34	0.29	0.33	0.32
Valine	1.00	0.99	1.02	1.02
Methionine	0.46	0.43	0.48	0.48
Isoleucine	0.90	0.84	0.90	0.84
Leucine	1.80	1.70	1.82	1.76
Tyrosine	0.75	0.71	0.76	0.72
Phenylalanine	1.06	0.97	1.05	1.00
Hydroxylysine	0.07	0.07	0.07	0.07
Ornithine	0.01	0.01	0.01	0.01
Lysine	1.44	1.47	1.48	1.44
Histidine	0.52	0.49	0.53	0.50
Arginine	1.25	1.15	1.25	1.16
Tryptophan	0.26	0.23	0.25	0.26
Total	20.54	19.35	20.64	19.77
Crude Protein	22.16	21.75	21.31	21.46
Moisture	12.70	12.38	12.36	12.50
Crude Fat	2.69	3.48	3.47	3.46
Crude Fiber	2.67	2.50	2.66	2.59
Ash	4.71	5.05	5.03	4.91

*Percentage of grams per 100 grams of sample

Table 3.6 Phase 5-10 Ingredient Composition of Control, 0.1% *Lactobacillus*-based Probiotic, 0.2% *Lactobacillus*-based Probiotic, and 0.2% *Bifidobacterium*-based Postbiotic (kg)

	Phase 5	**Phase 6**	**Phase 7**	**Phase 8**	**Phase 9**	**Phase 10**
Corn	632	676	727	786	812	871
Soybean Meal	229	186	137	119	107	98
DDGS	100	100	100	62	50	-
Corn Oil	6.0	5.7	10.2	9.5	9.0	4.5
Limestone	10.5	10.5	5.5	4.2	4.5	8.5
Monocalcium Phosphate 21%	4.0	3.7	5.0	5.0	5.0	4.0
Salt	5.0	5.0	5.0	5.0	5.0	5.0
Lysine HCL	5.4	5.1	3.3	3.5	3.0	3.2
Threonine PRO 80%	2.2	2.1	1.9	1.6	1.5	1.2
DL-Methionine-99%	1.7	1.2	1.0	0.5	0.4	0.1
PGF VTM[1]	1.0	1.0	1.0	1.0	1.0	1.0
L-Valine	0.4	0.2	0.4	-	-	-
L-Tryptophan	0.5	0.4	0.2	0.4	0.3	0.3
Total	1000	1000	1000	1000	1000	1000

[1]Provided per kilogram of the diet: 1,998 FTU phytase, 3,522 IU vitamin A, 1,101 IU vitamin D3, 22 IU vitamin E, 3.0 mg vitamin K3, 26.4 mg niacin, 17.6 mg pantothenic acid, 5.2 mg riboflavin, 23.8 ug vitamin B12, 30 mg Mn from manganous oxide, 100 mg Zn from zinc hydroxychloride, 80 mg Fe from ferrous sulfate, 12 mg Cu from copper chloride, 0.40 mg I from ethylenediamine dihydroiodide, and 0.30 mg Se from sodium selenite. Micronutrients: (Copper Chloride at 0.2 kg)

Table 3.7 Proximate Analysis Composition of Diet Phases 5-8

	Phase 5	Phase 7	Phase 8
Taurine	0.27	0.29	0.28
Hydroxyproline	0.03	0.02	0.03
Aspartic Acid	1.60	1.30	1.09
Threonine	0.86	0.62	0.52
Serine	0.75	0.63	0.52
Glutamic acid	3.01	2.43	2.06
Proline	1.09	0.84	0.77
Lanthionine	0.00	0.00	0.00
Glycine	0.70	0.58	0.50
Alanine	0.93	0.74	0.67
Cysteine	0.27	0.24	0.22
Valine	0.84	0.66	0.59
Methionine	0.41	0.26	0.25
Isoleucine	0.75	0.59	0.53
Leucine	1.61	1.26	1.16
Tyrosine	0.63	0.52	0.47
Phenylalanine	0.90	0.72	0.64
Hydroxylysine	0.06	0.07	0.05
Ornithine	0.01	0.01	0.01
Lysine	1.26	0.99	0.90
Histidine	0.45	0.36	0.32
Arginine	0.99	0.83	0.69
Tryptophan	0.23	0.16	0.16
Total	17.65	14.12	12.43
Crude Protein	19.24	14.97	13.31
Moisture	12.80	13.87	13.95
Crude Fat	1.86	2.02	2.04
Crude Fiber	2.49	2.09	1.80
Ash	4.56	3.15	2.86

*Percentage of grams per 100 grams of sample

Table 3.8 Average body weight (BW, kg) of pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.1% (0.1% LacPro), or 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

	Control	**0.1% LacPro**	**0.2% LacPro**	**0.2% BifPos**	**SEM**	***P*-value**
Day 0	6.0	6.1	6.2	6.1	0.1	0.64
Day 10	7.5	7.6	7.6	7.5	0.1	0.95
Day 21	11.3	11.2	11.4	11.3	0.2	0.93
Day 47	27.3	27.1	27.3	27.4	0.4	0.96
Day 70	48.5	48.8	48.7	49.1	0.6	0.92
Day 105	84.3	84.1	84.3	84.8	1.0	0.96
Day 135	114.5	114.6	114.1	114.2	1.3	0.99

Table 3.9 Average daily gain (ADG, g/d) of pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.1% (0.1% LacPro), or 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

	Control	**0.1% LacPro**	**0.2% LacPro**	**0.2% BifPos**	**SEM**	***P*-value**
ADG, g/d						
D 0 to 10	147.53	142.70	134.63	133.78	6.13	0.84
D 10 to 21	326.95	311.30	327.24	322.74	6.10	0.78
D 21 to 47	617.72	617.52	616.84	625.83	4.95	0.91
D 47 to 70	926.51	948.28	933.96	941.30	9.21	0.89
D 70 to 105	1020.86	1005.86	1015.61	1020.11	8.65	0.92
D 105 to 135	994.61	1006.03	989.60	979.40	10.50	0.84

Table 3.10 Average daily feed intake (ADFI, g/d) of pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.1% (0.1% LacPro), or 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos).

	Control	**0.1% LacPro**	**0.2% LacPro**	**0.2% BifPos**	**SEM**	***P*-value**
ADFI, g/d						
D0 to 10	153.62[b]	176.26[a]	175.13[a]	171.64[a]	3.38	0.05
D10 to 21	553.60	590.11	568.30	584.00	14.04	0.80
D21 to 47	960.62	916.27	932.38	908.26	11.05	0.36
D47 to 70	1684.63	1745.74	1645.82	1731.07	26.02	0.53
D70 to 105	2365.07	2364.43	2380.40	2293.72	31.45	0.78
D105 to 135	2403.50	2398.90	2394.34	2381.32	30.34	0.99

*ab within a row, means lacking a common superscript differ ($P<0.05$)

Table 3.11 Gain to feed ratio (G: F) of pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.1% (0.1% LacPro), or 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

	Control	**0.1% LacPro**	**0.2% LacPro**	**0.2% BifPos**	**SEM**	***P*-value**
G:F						
D0 to 10	0.96	0.82	0.77	0.80	0.03	0.51
D10 to 21	0.60	0.56	0.59	0.56	0.02	0.69
D21 to 47	0.64	0.68	0.66	0.69	0.01	0.28
D47 to 70	0.55	0.55	0.57	0.55	0.01	0.76
D70 to 105	0.43	0.43	0.43	0.45	0.01	0.63
D105 to 135	0.42	0.42	0.42	0.41	0.01	0.97

Table 3.12 Hot carcass weight (HCW, kg) of pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.1% (0.1% LacPro), or 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

	Control	**0.1% LacPro**	**0.2% LacPro**	**0.2% BifPos**	**SEM**	***P*-value**
HCW wk 1	97.7	97.4	96.6	98.2	1.1	0.81
HCW wk 2	98.7	99.7	98.3	96.5	1.1	0.23
HCW wk 3	99.2	99.7	98.3	101	1.3	0.61
HCW wk 4	102^{x}	101^{x}	97.4^{y}	99.0^{x}	1.4	0.08
HCW overall	99.5	99.5	97.7	98.6	0.8	0.32

*ab within a row, means lacking a common superscript differ ($P<0.05$)

Each week included 4 pigs per treatment pen, reaching 40 pigs per treatment each week.

Table 3.13 Mortality of pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.1% (0.1% LacPro), or 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

TRT	Frequency	Percent	Cumulative Frequency	Cumulative Percent
CON	11	26.8	11	26.8
0.1% LacPro	13	31.7	24	58.5
0.2% LacPro	9	22.0	33	80.5
0.2% BifPos	8	19.5	41	100

Table 3.14 Histological analysis from ileum and jejunum tissue at Day 10 of pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.1% (0.1% LacPro), or 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

	Control	**0.1% LacPro**	**0.2% LacPro**	**0.2% BifPos**	**SEM**	***P*-value**
Ileum (µm)						
Villi height[1]	255.0	288.5	289.0	296.0	16.8	0.33
Crypt depth	263.8	274.4	241.3	252.8	14.0	0.41
VCR[2]	0.99[c]	1.0[b]	1.2[a]	1.2[a]	0.05	0.02
Jejunum (*µ*m)						
Villi height	341.6	350.1	385.5	348.5	17.9	0.36
Crypt depth	314.0	303.1	290.5	285.8	10.9	0.30
VCR	1.11	1.17	1.33	1.22	0.06	0.15

*a-c within a row, means lacking a common superscript differ ($P<0.05$

[1] 10 samples for each treatment from 20 measurements per pig

[2] VCR; Villus height to crypt depth ratio

Table 3.15 Mean relative abundance (%) of main bacterial groups in feces collected on Day 10 from pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

	Control	**0.2% LacPro**	**0.2% BiffPos**	***P*-value**
Lachnospiraceae	57.1	50.1	48.3	0.16
Lactobacillaceae	3.5[b]	10.4[a]	10.8[a]	0.03
Streptococcaceae	0.2	0.1	0.3	0.99
Clostridiaceae 1	5.7	4.4	3.9	0.88
Erysipelotrichaceae	6.2	8.2	10.1	0.55
Ruminococcaceae	9.8	8.5	9.1	0.95
Other Firmicutes	9.9	7.7	9.4	0.65
Prevotellaceae	1.1	3.4	1.5	0.88
Other Bacteroidetes	0.6	0.6	2.1	0.90
Actinobacteria	0.9	1.2	1.5	0.53
Unclassified Bacteria	4.3	5.4	2.6	0.88
Other Phyla	0.6	0.2	0.5	0.69

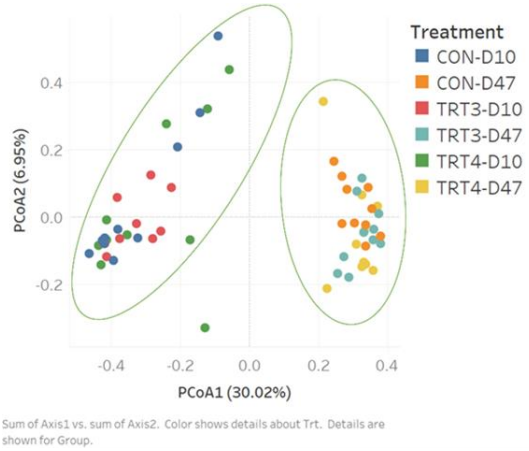
*ab within a row, means lacking a common superscript differ ($P<0.05$)

Table 3.16 Mean relative abundance (%) of main bacterial groups in feces collected on Day 47 from pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos)

	Control	**0.2% LacPro**	**0.2% BifPos**	***P*-value**
Lachnospiraceae	12.7	14.0	12.6	0.76
Lactobacillaceae	25.8^{y}	33.6^{x}	36.2^{x}	0.08
Streptococcaceae	26.4^{x}	20.7^{y}	14.9^{y}	0.07
Clostridiaceae 1	8.6	8.4	9.9	0.61
Erysipelotrichaceae	2.2	3.8	3.5	0.80
Ruminococcaceae	6.5	5.1	6.1	0.28
Other Firmicutes	7.1	5.3	6.6	0.33
Prevotellaceae	7.2	4.4	6.0	0.30
Other Bacteroidetes	0.3	0.5	0.3	0.23
Actinobacteria	1.4	1.8	0.5	0.89
Unclassified_Bacteria	1.3	2.0	2.5	0.11
Other Phyla	0.7	0.4	0.9	0.46

*xy Within a row, means lacking a common superscript are tendencies ($0.05 \leq P \geq 0.10$)

Figure 3.1 Biodiversity analysis from feces collected on Day 10 and Day 47 from pigs fed corn-soybean meal-based diets with no dietary inclusion of probiotic or postbiotic (Control), Control plus 0.2% dietary inclusion of *Lactobacillus*-based probiotic (0.2% LacPro), or Control plus 0.2% dietary inclusion of *Bifidobacteria*-based postbiotic (BifPos).



FINAL DISCUSSION

The primary focus of this thesis was to assess the effects of a different protein source, in this case, CFP, and evaluate the effects of including *Lactobacillus*-based probiotics at two levels (0.1% and 0.2%) and a *Bifidobacterium*-based postbiotic on nursery pig's growth performance, gut health and in the second study microbial populations were also evaluated. For the first study, we hypothesized that increasing levels of CFP would negatively affect growth performance and greater permeability in the gut. In the second study, it was hypothesized that the addition of *Lactobacillus*-based probiotics and *Bifidobacterium*-based probiotics would increase feed intake, increase beneficial bacteria in the GIT, and improve VH and CD in the jejunum and ileum.

Weaning could negatively impact pigs due to various stressors mentioned throughout the literature review. However, by implementing dietary intervention strategies, many stress-related issues faced by weaned pigs, such as reduced feed intake and growth, changes in the structure and function of the small intestine, decreased intestinal barrier function, reduced feed intake, and lower body weight gain (Tang et al., 2022), can be improved. Therefore, assessing gut permeability through the Differential sugar absorption test and measuring histological data can give us a status of the health of the GIT. Evaluating growth performance can also give us an on-farm assessment of the health status of the pig.

Regarding growth performance, our findings in the first study resulted in pigs fed all three inclusion levels of CFP having no difference in BW throughout the trial. In the second trial, results regarding BW also showed no treatment effects. However, ADFI results in both studies demonstrated a positive impact of the treatment diets.

Supplementing pigs with 4% and 8% inclusion of CFP in the diet in the first two weeks after weaning (Phase 1), we had similar responses with pigs fed only soy protein concentrate. The findings from our second study primarily showed an improved feed intake for pigs fed 0.1%, 0.2% *Lactobacillus*-based probiotics, and 0.2% *Bifidobacteria*-based postbiotic during the first ten day of supplementation. Low feed consumption during the first three day post-weaning may disrupt nutrient intake, body weight loss, and intestinal disturbances that lead to a high occurrence of GIT inflammation (McCracken et al., 1999); some pigs may recover from this reduction, but it may lead to morbidity and mortality (Wensley et al., 2021). Feed intake is a key determinant of performance and health status of weanling pigs. It is typical for weanling pigs to exhibit no or minimal feed intake, falling below their maintenance energy needs, during the initial 3 to 5 day post-weaning (Fabá et al., 2024). The lack of nutrients in the intestinal lumen results in disturbances, including intestinal villus atrophy, reduced enzyme activity, nutrient absorption, and increased intestinal permeability (Fabá et al., 2024). The importance of including CFP in nursery pig diets is due to its greater digestible amino acid content than DDGS (Acosta et al., 2021). Amino Acids are valuable for a newly weaned pig and essential for the maturation and development of the intestine (Mou et al. 2019). Proper amino acid balance is crucial for weaned piglets' systemic homeostasis and intestinal physiology (Mou et al., 2019). The intestine is a crucial organ involved in the digestion, absorption, and metabolism of dietary nutrients. Additionally, it functions as a physical barrier, engaging with a complex external environment (Gao et al., 2020).

In chapter three, we evaluated the changes in the microbial population by including *Lactobacillus*-based probiotics and *Bifidobacteria*-based postbiotics. As the literature mentions, adding probiotics and postbiotics has numerous roles in maintaining a

healthy GIT in weaned pigs. Supplementing LAB increases the relative abundance of *Lactobacillus* and *Bifidobacterium spp*., decreases *E. coli,* and enhances the production of short-chain fatty acids in the gut of the weaned pig (Wang et al., 2021). Probiotic-produced SCFAs contribute to defending against pathogenic microbial colonization by downregulating the GIT pH (Wang et al., 2021). Additionally, SCFAs enhance gut barrier function by providing energy to intestinal epithelial cells (D'Souza et al., 2017). Another relevant result of our research was that the histological analysis from ileal tissue at day 10 of pigs fed 0.2% *Lactobacillus*-based probiotic and 0.2% *Bifidobacteria*-based postbiotic had a greater VCR compared to pigs fed the control and 0.1% *Lactobacillus*-based probiotic. These results are similar to Sun et al. (2021), who fed a mix of probiotics (*L. acidophilus, L. casei, B. thermophilum, and E. faecium*) at a concentration of 0.25 x 10^8 CFU/g for each strain to weaned pigs for 25 day. For nursery pigs to have a greater VH, CD may signify a better nutrient absorption capacity, which implies a healthier GI tract; therefore, the stressors that negatively affect the weaned pigs are reduced (Su et al., 2022). Overall, the inclusion of Lactobacillus-based probiotics and Bifidobacteria-based postbiotics did not affect growth performance when compared to pigs fed only control diet. The impact of probiotics and postbiotics may vary with the levels of inclusion which I would recommend to evaluate, as well as to measure different parameters such as diarrhea incidences and medication use to really see if it is viable to include probiotics and or postbiotics in nursery pig diets.

APPENDIX

Statistical Models

Chapter 2

1. Growth Performance

```
proc glimmix data=growth;
class diet;
model BW0= diet/ddfm=kr;
lsmeans diet/pdiff;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

*This model was used for BW, ADG, ADFI, G: F days 7, 14, 21, 28, 35, and 42.

Chapter 3

1. Growth Performance

```
proc glimmix data=growth;
class diet;
model BW0= diet/ddfm=kr;
lsmeans diet/pdiff;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

*This model was used for BW, ADG, ADFI, G: F days 10, 21, 47, 70, 105, and 135

2. Histological Analysis

```
proc glimmix data=growth;
class trt;
model Vh= trt/ddfm=kr;
lsmeans trt/pdiff;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

*This model was also used for crypt depth, and villus heigh to crypth depth ratio

3. Hot Carcass Weight

```
proc glimmix data=growth;
class diet;
model wk1= diet/ddfm=kr;
lsmeans diet/pdiff;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;
```

*This model was also used for week 2, 3, 4, and overall HCW

4. Microbiome Mean Relative Abundance

```
setwd("C:/Users/DELL_X/Desktop/ADM trial march 2022/Microbiome analysis")
data<-read.csv("TaxonomyD10.csv")
head(data)
data_long<-melt(data,id.vars = "Group")
head(data_long)
colnames(data_long)[1]<-"Taxa"
colnames(data_long)[2]<-"Group"
data_long$Taxa<-as.factor(data_long$Taxa)
Taxa<-levels(data_long$Taxa)
Taxa
```

```
data_long$Group<-gsub("\\.([0-9][0-9])|\\.([0-9])","",data_long$Group)
data_long$Group<-as.factor(data_long$Group)
count=c(0)
for (i in Taxa){
 count=count+1
 print(paste("Count=",count,"; by Ranch"))
 local_data<-data_long[which(data_long$Taxa==i),]
 print(paste("Taxa:",i))
 print(paste("kruskal test:"))
 ktest<-kruskal.test(local_data$value~local_data$Group)
 print(paste("kruskal test P-value:",ktest$p.value))
 print(paste("Is significant?", ifelse(ktest$p.value<=0.05, "Yes", "No")))
 print(paste("Wilcox Test:"))
 if(ktest$p.value<0.05){
  P<-pairwise.wilcox.test(local_data$value,local_data$Group, p.adjust.method="none")
  PT = P$p.value
  PT
  PT1 = fullPTable(PT)
  PT1
  if(sum(is.nan(PT1))==0){
   PT1 = multcompLetters(PT1,
                         compare = "<",
                         threshold = 0.05,
                         Letters=letters)
   print(PT1)
  } else { print(PT1)
```

*This model was also used for the mean relative abundance for day 47.

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Final Discussion

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