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Evaluation Of Yeast Postbiotic in Sow Diets on Sow and Offspring Performance and Microbial Succession

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EVALUATION OF YEAST POSTBIOTIC IN SOW DIETS ON SOW AND OFFSPRING PERFORMANCE AND MICROBIAL SUCCESSION

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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.

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Chapter 1

1.0 Literature Review

1.1 Introduction

In commercial swine production, economics drive production decisions and increasing pig growth performance and sow reproductive performance have been a continual push as a means to maintaining profitability. Two different articles published by National Hog Farmer, one in 2017 and one in 2021, report the volatility of producing pork in a global market and how rapidly operating costs and producer perceptions change with an encouraging input cost report in 2017 (Kerns, 2017). This was followed by an article predicting a steep rise in input costs published in 2021 (Farmer sentiment weakens, 2021). In an article published by Mike Brumm from University of Nebraska, the increase in growing-finishing pig growth performance in recent years was explored and showed major increases in average market weight and daily gain coupled with decreases in days to market and feed to gain ratio from 1980 to 2001 (Brumm, 2002). This trend has continued to 2020 according to data from a report of hog production in Ireland published by Teagasc (National Pig Herd Performance Report 2020, 2021). These increases in finishing pig performance have helped to maintain economic viability for commercial swine producers; however, before the growing-finishing period (i.e., suckling or nursery period) there are many areas to improve to continue profitable production.

According to reports by the USDA, the numbers of swine breeding stock have stayed somewhat stable from 1990-2019; however, the number of marketable hogs has risen quite substantially (Swine 2000, 2005; Quarterly Hogs and Pigs, 2019). The rise of highly prolific sows through an increase in number of piglets born has corresponded with

the development of highly efficient and lean offspring which combine to place massive nutritional and metabolic demands on the modern sow (Kim et al., 2013; Tokach et al., 2019). While these improvements have contributed to economic sustainability in the pork industry, they have also come with negative consequences and new challenges such as an increase in piglet mortality, lower birth weights, and increased within-litter variation (Knol et al., 2002; Quesnel et al., 2008; Foxcroft et al.). Focusing on reproductive performance along with piglet survivability and vitality will result in pigs better able to manage stress at weaning and throughout the nursery and growing-finishing period to increase the pounds of pork produced per sow per year.

1.2 Weaning Stress

Weaning has been well documented as the most stressful period in the life of a pig. In nature, weaning of piglets from the sow is a process which usually occurs between 10 and 12 weeks of age; however, in today's modern pig production, weaning usually occurs between 14 and 30 days of age (Moeser et al., 2017). This process of early weaning coincides with major development of the gastrointestinal tract and immune system in the piglet along with a natural decrease in passive immunity from the sow which compounds the stress on the piglet from maternal separation, transportation, mixing, and establishment of a new social hierarchy (Moeser et al., 2017). In a review by Campbel et al. (2013), the biological signs of early weaning stress are well explored including changes in performance and feed intake, changes in gut structure and function, and the increase in gut inflammation. Weaning often encompasses physical relocation of piglets and separation from the sow which induces acute stress; however, the larger change for the digestive system and microbiome is adapting from highly digestible milk

to a solid, less digestible diet (Campbell et al., 2013). This adaptation, which results in lower piglet feed intake for a period of time, has been shown to contribute to piglet weight loss, intestinal inflammation, gastrointestinal tract structure and function, and days to market (Kats et al., 1992; McCracken et al., 1999; le Dividich and Sève, 2000; Spreeuwenberg et al., 2001).

A variety of strategies have been employed to ease the nutritional transition around weaning to varying degrees of success including but not limited to pre-weaning milk replacer supplementation (Greef et al., 2016), pre-weaning creep feed supplementation (van der Meulen et al., 2010; Middelkoop et al., 2020), varying photoperiod length post-wean (Niekamp et al., 2007), and adjusting weaning age (Colson et al., 2006; Jarvis et al., 2008; van der Meulen et al., 2010). Many of these husbandry practices utilized around weaning are an attempt to stabilize and improve gut health in preparation for, or in reaction to, the environmental and biological stresses of weaning (Campbell et al., 2013; Jayaraman and Nyachoti, 2017). Recently, probiotics (Hayakawa et al., 2016; Gresse et al., 2017; Xiang et al., 2020), prebiotics (Jiao et al., 2014; Gresse et al., 2017), and postbiotics (Holanda et al., 2020; Hsun Ho, 2020) have been explored as popular methods to alleviate the effects of weaning stress.

1.3 Probiotics, prebiotics, and postbiotics

1.3.1 Probiotics

In 2001, a group of international scientists convened to rework and establish a working definition for the term “probiotic” based on increasing interest in research regarding probiotics (Hill et al., 2014). This definition, which states that probiotics are

"live microorganisms which when administered in adequate amounts confer a health benefit on the host", is still being utilized today (FAO and WHO, 2006). The public database, PubMed, has indexed more than 8,000 research articles utilizing the word probiotic from 2001-2014 and this field of research has continued to expand (Hill et al., 2014). Probiotic bacteria which often includes members of lactic acid bacteria such as lactobacilli, enterococci, and bifidobacteria have been researched heavily for their effect on a myriad of health, developmental, and growth outcomes (Ouwehand et al., 2002). Microorganisms used in traditional bacterial probiotics are most often derived from *Lactobacillus, Streptococccus, Enterococcus, Bacillus, Clostridium,* and *Bifidobacterium* species*, and Escherichia coli* strains which are largely Gram positive, lactic acid producing microbes (T et al., 2017). *Lactobacilli* and *Bifidobacteria* are currently the most deeply understood probiotic bacteria and knowledge is vague concerning specific effects of other species. *Lactobacilli* aid in digestion of lactose, reduce constipation, reduce host infections by pathogens such as *Salmonellae,* and help relieve irritable bowel syndrome treatment (Czerucka et al., 2007). *Bifidobacteria* may stimulate the immune system, produce B vitamins, inhibit pathogen growth, reduce blood ammonia and cholesterol, and reestablish normal flora post antibiotic treatment (Czerucka et al., 2007). Multiple strains of yeast are well documented to have similar probiotic effects on health, growth, and development (van Heugten et al., 2003; Nunes et al., 2012; Broadway et al., 2015). When administered via food applications, probiotic microorganisms must possess a variety of characteristics such as high viability, stability during storage, resistance to gastric acidity, resistance to bile and pancreatic enzymes, adherence to intestinal mucosal cells, and colonization capacity to maintain efficacy (Ayichew et al., 2017). Although

certain strains of yeast and bacteria may be considered probiotic microorganisms, their inherent differences imply a different mechanism and site of action when applied as probiotics (Broadway et al., 2015). Probiotic bacteria may exert their positive effect on the host through several potential mechanisms including producing substances which inhibit or kill pathogenic organisms, competing with pathogenic organisms for adhesion sites or nutritional sources, neutralizing bacterial toxins, and modulating the host immune system (T et al., 2017). Probiotic yeast possess similar, although slightly unique mechanisms of action in the host by acting through inactivation of bacterial toxins, modifying host cell signaling to induce a protective effect against pathogenic bacteria, increasing secretion of IgA and subsequent receptors in the small intestine, stimulating brush border membrane enzyme activity, and reducing inflammatory responses in the intestine (Broadway et al., 2015). Since the inception of probiotics in animal agriculture, the emphasis of research and application has been largely on improving animal performance through supplementation. However, with an ever-increasing focus on reducing antibiotic use in commercial pig production, researchers are exploring the possibility that probiotics may partially replace use of antibiotics (van Heugten et al., 2003; Reid, 2006; Nunes et al., 2012; Broadway et al., 2015).

1.3.2 Prebiotics

Understanding how to effectively manipulate the microbiome, especially probiotic microorganisms, is essential to achieve beneficial outcomes. The importance of inclusion of oligosaccharides as part of dietary fiber in sow diets has been realized due to their unique physiological effects especially on the microbiome (Slavin, 2013). These indigestible carbohydrates are known as prebiotics or “a selectively fermented ingredient

that allows specific changes both in composition and/or activity in the gastrointestinal microflora that confers benefits on host well-being and health" (Gibson et al., 2004; Slavin, 2013). To be considered a prebiotic, dietary carbohydrates must possess distinct characteristics such as resistance to gastric acidity, resistance to hydrolysis by mammalian enzymes, resistance to gastric absorption, ability to be fermented by intestinal microflora, and fosters selective stimulation of growth/activity of intestinal microbes that are beneficial for the host (Roberfroid, 2007). Prebiotic carbohydrates include resistant starch, non-starch polysaccharides, and oligosaccharides, but oligosaccharides are the primary prebiotic compounds (Manning and Gibson, 2004; Slavin, 2013). In monogastric animals, resistance to digestion is imperative as these compounds much reach the large intestine where a vast majority of the gut microbiota reside to ferment carbohydrate substrates (Roberfroid, 2007; Davani-Davari et al., 2019; F et al., 2019)

Dietary prebiotics compose the chief source of energy for growth of the microbiome where main end products of carbohydrate fermentation in the large intestine are short chain fatty acids (SCFA) such as acetate, propionate, and butyrate. These short chain fatty acids can be metabolized to provide energy for the host or local microbiota (Manning and Gibson, 2004; Davani-Davari et al., 2019). The end products of fermentation such as SCFA and peptidoglycans elicit several effects across the host which are beneficial for the host such as affecting T-helper 2 in the airways and macrophages, impacting dendritic cells in bone marrow, decreasing colon pH, and stimulating the innate immune system against pathogenic organisms (Davani-Davari et al., 2019). Probiotic *Lactobacilli* and *Bifidobacteria* are the most common targeted

genera for proliferation by prebiotics due to their known benefits and preference for oligosaccharides (Slavin, 2013).

The entire mechanism for selective stimulation of gut microbiome is not fully elucidated; however, factors contributing to this mechanism are being pieced together slowly. Molecular weight of prebiotics likely resulting from different chain lengths play a large role in selective stimulation; for example, xylans (longer) are not selectively fermented whereas xylo-oligosaccharides (shorter) are thought to be specifically fermented by certain microorganisms (Manning and Gibson, 2004). Chain-length is important for distinguishing bacterial species capable of fermenting specific prebiotics (Manning and Gibson, 2004). For example, only a few species can ferment longer chain prebiotics, but most prebiotics are short chained and fermented by a larger number of microorganisms (Manning and Gibson, 2004; Davani-Davari et al., 2019). Growth of microorganisms stimulated by prebiotics can further permeate the colon due to slower fermentation with longer chained oligosaccharides and therefore have more impact on the distal colon than shorter chain prebiotics (Manning and Gibson, 2004; Davani-Davari et al., 2019). Cross-feeding is a phenomenon where a by-product of fermentation of a complex prebiotic is a substrate for another microorganism which is targeted when utilizing long chain prebiotics (Davani-Davari et al., 2019). Prebiotics have many diverse applications and exert a multitude of effects on the microbiome which present many possible nutritional, health, and developmental outcomes.

1.3.3 Postbiotics

Probiotic microorganisms exert their effects on the host through a variety of mechanisms, most of which rely on compounds or substances released from the

microorganism. These components released by live microorganisms or upon microorganism death are termed postbiotics. Postbiotics are defined as a "preparation of inanimate microorganisms and/or their components that confer a health benefit on the host" (Salminen et al., 2021). In practicality, postbiotics encompass a wide variety of molecules such as cell-free supernatants, exopolysaccharides, enzymes, cell wall fragments, SCFAs, bacterial lysates, and metabolites produced by gut microbiota (lipoteichoic acids and other polysaccharides) (Aguilar-Toalá et al., 2018; Żółkiewicz et al., 2020). Postbiotics are often considered to be inactivated microorganisms or components of once viable microorganisms, it is hypothesized that efficacy of effector molecules is increased if the cellular structure of the postbiotic is conserved as the cell wall protects against rapid digestive enzyme degradation and immune attacks (Salminen et al., 2021). Although the effect of postbiotics on the microbiota may be temporary in comparison to probiotics (probiotics are living and can continue to elucidate an effect over a period of time), postbiotics offer many new avenues for microbial application by avoiding many of the difficulties working with live microorganisms such as colonization efficiency, keeping microorganisms viable and stable in high enough concentrations to achieve a benefit, improving shelf-life, and simplifying packaging and shipping (Wegh et al., 2019). Postbiotics are derived mainly from *Lactobacillus* and *Bifidobacterium* strains because of their proven efficacy to elicit a positive outcome on the host; however, other strains of bacteria such as *Streptococcus* and *Faecalibacterium* and some strains of yeast have potential for utilization as postbiotics (Aguilar-Toalá et al., 2018).

In the host, postbiotics prompt a myriad of effects, which include modulating microbiota, enhancing gut epithelial barrier function, modulating host immune responses

locally and systemically, moderating systemic metabolism, and a variety of health and recovery impacts during disease or health challenges (Żółkiewicz et al., 2020; Salminen et al., 2021). Immunomodulation occurs through controlling production and release of multiple interleukins as well as decreasing inflammation during exposure to postbiotics (Aguilar-Toalá et al., 2018; Żółkiewicz et al., 2020). Postbiotic compounds shift the microbial composition of the gut and improve intestinal barrier function through lactic acid production and distribution, competition for binding sites in the intestine, and competitively binding to receptors required for pathogenic bacteria (Żółkiewicz et al., 2020; Salminen et al., 2021). Use of postbiotics provides another tool, in concert with probiotics and prebiotics, for control and manipulation of the microbiome to provide beneficial outcomes for the host.

1.4 Yeast Biotics

1.4.1 Yeast Probiotics

Early exploration into the field of probiotics revolved around bacteria, most commonly lactic acid producing bacteria, and has since blossomed into a field investigating many different types of microorganisms including yeasts. Probiotic yeasts, generally *Saccharomyces cerevisiae,* can improve feed efficiency and digestibility, reduce animal pathogen load, enhance animal performance and health, and potentially reduce negative environmental impacts (Haldar et al., 2011; Cheng et al., 2014; Ogbuewu et al., 2019; Elghandour et al., 2020). Co-supplementation or co-culturing probiotic yeasts with probiotic lactic acid bacteria may enhance survivability of dietary probiotic lactic acid bacteria in the host (Liu and Tsao, 2009). Prominent features of probiotic yeasts include inherent antibiotic resistance, *anti*-mycotoxigenic and phytate degrading

abilities, and health promotion in the host (Sadeghi et al., 2022). Consistent with other studies evaluating probiotics, the outcome of dietary supplementation with live yeast produces inconsistent results on animal performance with some researchers reporting improved growth performance and others reporting no differences (Kornegay et al., 1995; Medina et al., 2002; van der Peet-Schwering et al., 2007). Observed variations are likely the product of varying applications in types and doses of yeast as well as feed composition, animal anatomy and physiological status (Elghandour et al., 2020). Interestingly, yeasts flow through the digestive tract as viable microorganisms and are generally not found adhered to the cells of the gastrointestinal tract; however, these yeasts act through microbial antagonistic stimulation of the host immune system, removal of pathogens, and increased activity of specific bacterial enzymes (Elghandour et al., 2020). Probiotic *Saccharomyces cerevisiae* are rich in digestible proteins, B-vitamins, magnesium, and zinc (Elghandour et al., 2020). The yeast cell wall, comprised mainly of mannans and β-glucans, provide much of the immunological basis for how yeast affects the immune system of the body (Rodrigues et al., 2000; J. Li et al., 2006). Several extensive reviews outline proposed mechanisms for the myriad of effects yeast has on the body including immunomodulation, metabolic effects, microflora effects, and physiological changes (Ogbuewu et al., 2019; Elghandour et al., 2020). Live probiotic yeasts have demonstrated a number of benefits for the host; however, prebiotic and postbiotic applications of lysed yeast cells may underlie many of the mechanisms proposed for live yeasts whereby yeasts confer their benefit which creates many possibilities for novel methods to administer these advantages to the host (Chan and Liu, 2022). Using yeast culture as a postbiotic may provide a viable alternative to probiotic

yeasts in animal populations with immature or compromised immune systems due to a possibility of fungal infection in these populations (Imre et al., 2021; Chan and Liu, 2022).

1.4.2 Yeast Culture

Co-products from yeast fermentation-based production of ethanol and beer have long been recognized for their value in animal nutrition; however, characterization of the contribution of yeast to the gut microbiome composition has not been well defined (Böttger and Südekum, 2018; Shurson, 2018). Yeast culture, defined as a dried mixture mostly containing various metabolites from yeast fermentation and possibly a small amount of live yeast cells, may exemplify a mechanism for conferring benefits to the host and their microbiome through a postbiotic/prebiotic treatment (van der Peet-Schwering et al., 2007; Shen et al., 2009a). While the specific composition of postbiotics may vary in levels of certain metabolites, most yeast postbiotics likely contain standard products of yeast metabolism and structural components including bioactive oligosaccharides and peptides, carotenoids, polyphenols, β-glucans, GABA, and prebiotic oligosaccharides (Rai et al., 2019; Sadeghi et al., 2022). Cell wall constituents of yeast, including β-glucan, mannoprotein, and chitin, are likely modulators of toxin and pathogen adsorption by yeast postbiotics leading to decreased disease incidence and better immune function (Fortin et al., 2018; Pereyra et al., 2018; Liu et al., 2021; Chan and Liu, 2022). B-glucans in particular have been shown to have a potent response on stimulation of the immune system and serve as antioxidants (Jaehrig et al., 2007; Smith et al., 2016). Mannan portions of the cell wall likely serve as prebiotic oligosaccharides and may act as antioxidants (Al-Manhel and Niamah, 2017; Galinari et al., 2018; Rai et al., 2019).

Polyamines, as part of the metabolite mixture derived from yeast cells, may improve macronutrient digestion by enhancing expression of intestinal digestive enzymes and nutrient uptake transporters, while acetic and decanoic acids secreted by yeasts may inhibit several gut opportunistic pathogens (Pais et al., 2020; Suchodolski et al., 2021; Chan and Liu, 2022). Multiple enzymes and effector molecules derived from probiotic yeasts elicit a multitude of outcomes in the host related to gut health and immune function (Chan and Liu, 2022). Several nutraceutical compounds found in yeast extracts, including γ-Aminobutyric acid (GABA), folate, glutathione, and carotenoids, provide opportunities for improved health by reducing oxidative stress, neutralizing reactive oxygen species, and providing cofactors for biochemical reactions (Rai et al., 2019). The many positive outcomes which result from supplementing diets with live yeasts as well as inactivated or dead yeast cells make investigation into supplementation of these products in all stages of swine production a priority for advancement of the commercial swine industry.

1.5 Swine Microbiome Composition and Function

1.5.1 Swine Microbiome Composition

Characterizing the composition of the porcine microbiome presents many challenges due to its constant adaptation as the animal develops and experiences different environmental and health statuses. Researchers must characterize the microbiome to understand the mechanisms behind the numerous outcomes elicited by the microbiome throughout growth and development of the pig. There are several major drivers of change in the gut microbiome during the life of a pig. Age of the pig has important influences on longitudinal change in the gut profile. Diversity of the microbiome increases with time

and bacterial communities of the duodenum, jejunum, and ileum possess less microbial variety than communities of the cecum and colon as the pig ages(de Rodas et al., 2018). While there are many variations in specific microbial compositions of the gut based on environment, health status, and management, the gut microbiome composition of the early postnatal pig appears to be dominated by *Clostrideaceae* and *Enterobacteriaceae* species with a secondary colonization of *Lactobacillaceae* species in the first few days post-parturition, whereas, in contrast, the post-weaning microbiome is characterized by rises in *Prevotella* and *Lactobacillus* species with a decrease in *Bacteroidaceae* species (Petri et al., 2010; de Rodas et al., 2018). The pigs gut microbiome diversifies over the first few weeks of life until later in life when *Firmicutes* and *Bacteroidetes* species account for many of the species in post-weaning and finishing pigs due to the anaerobic environment of the lower gut (Kim et al., 2011; Mach et al., 2015). While changes occur over time in the gut microbial community, many of those shifts may be a result of dietary changes and stress. The largest changes in the microbial community of the pig occur around the time of weaning characterized by massive increases in diversity as there is a shift from nursing to a solid plant-based diet (de Rodas et al., 2018; Aluthge et al., 2019; Nowland et al., 2019a). A recent review summarizes the genera of bacteria that dominate the gastrointestinal tract prior to weaning as *Bacteroides*, *Oscillibacter*, *Escherichia/Shigella*, *Lactobacillus*, and unclassified *Ruminococcaceae* genera and post-weaning as *Acetivibrio*, *Dialister*, *Oribacterium*, *Succinivibrio*, and *Prevotella* genera with an increase in diversity following weaning (Nowland et al., 2019b). The number and variety of bacterial species as well as the large changes in the microbial species of the swine gut microbiome over time speak to the many factors which may shift the microbial

composition of the pig. The ability of the microbiome to shift in composition and diversity with changes in the animal's health and environment underlines the importance of understanding how these microbial changes impact the disease status and growth of the animal.

1.5.2 Swine Microbiome Adaptations

There are specific changes in the microbiome in response to different dietary interventions, disease status, and environment of the pig which are quintessential to understand as a means to shift the microbiome in a way that will benefit the host. Though the main changes in the bacterial community due to the diet happen with the massive dietary change at weaning, small nutrient changes can impact the microbiome as well. For example, *Firmicutes* and *Bacteroidetes* dominate the ileal microbiota of growing pigs, but *Bacteroidetes* decreased with decreasing levels of dietary protein likely due to their proteolytic activity, and *Proteobacteria* and *Bacteroidetes* were most impacted by dietary protein levels (Qiu et al., 2018). Reducing indigestible protein in the diet decreased the prevalence of *Tenericutes* which may be associated with a higher health status while higher counts of *Lactobacilli* have been associated with an increase in dietary crude protein content (Wellock et al., 2006). *Bifidobacteria* are positively linked to dietary crude protein level (Peng et al., 2017). As previously discussed, dietary carbohydrates (i.e. prebiotics) also have great potential for impacting composition of the gut microbiome. Nutrition is one of the most fundamental tools for driving positive change in the swine microbiome whether the goal is to improve growth, health, or reproduction.

Another more recently discovered driver of change in the gut microbiome is stress. Weaning is the pinnacle of stress in a piglet's life in regard to growth, development, and health. Weaning often results in a reduction in growth likely due in part to increased intestinal permeability; however, there has been no reported research on the impact of housing-related stress (e.g. crate vs. pen gestation) on changes to the sow or offspring microbiome (Peng et al., 2017; Aluthge et al., 2019). Multiple studies have been completed in mice investigating the possibility of a bi-directional relationship between the microbiome and stress and show the possibility of altering the stress response of an animal by introducing different gastrointestinal tract (GIT) bacteria (Aluthge et al., 2019). The impact of the brain on gut function has been well established; however, the reverse relationship has been a topic of increasing interest in recent years. The magnitude of impact the microbial composition of the gut has on the brain is yet to be elucidated, but a strong link between gut microbiota and the stress response of the hypothalamic-pituitary-adrenal axis in the brain is well reviewed (Dinan and Cryan, 2012).

1.5.3 Gut-Brain Axis

The gut microbiota and brain communicate via many pathways including the immune system, tryptophan metabolism, the vagus nerve, and the enteric nervous system (Cryan et al., 2019). The magnitude of linkage between gut microbiota and the brain has been confirmed by demonstrating that brain, behaviour, and many health conditions were affected by complete absence of gut microbiota, administration of certain strains of bacteria, and administration of antibiotics (Cryan et al., 2019). The gut microbiota regulates and produces several neuroactive biomolecules which are either regulated by

the microbiome or produced from microbial degradation of fibers (Al-Khafaji et al., 2020). The extensive relationship of the gut-brain axis has been reviewed in detail by Cryan et al. (2019) and Al-Khafaji (2020); therefore, only a few highlights will be discussed here to underline the importance that a shift in microbiome can have on the brain. Production of SCFA by the gut microbiome have impacts on the brain by improving blood-brain barrier permeability and regulating catecholamine and dopamine synthesis, degradation, and transport (DeCastro et al., 2005; Braniste et al., 2014). Strains of *Lactobacillus* synthesize serotonin from tryptophan while administered antibiotics decrease gut microbial diversity and serotonin levels (ÖZOĞUL et al., 2012; Ge et al., 2017). Gut dysbiosis, characterized by losses of bifidobacteria, increased gram-negative bacteria, and decreased microbial diversity, is a typical early sign of neurodegenerative disorders and may participate in triggered central nervous system (CNS) disorders (Forsyth et al., 2011; Al-Khafaji et al., 2020). Dietary administration of probiotics, prebiotics, and postbiotics can shift the gut microbiome as explored above; therefore, the gut-brain axis also has a high potential to be modulated via nutrition.

1.5.4 Antibiotics

Development and utilization of antibiotics has produced a tremendous impact on commercial swine production with these compounds demonstrating their potential for disease treatment, disease control, disease prevention, and increased growth performance (O'Neill, 2014; Zeineldin et al., 2019). In recent years realization of the impact widespread antibiotic usage in commercial animal agriculture has on the microbiome of animals as well as the development and transfer of antimicrobial resistant genes from animal microorganisms to human microorganisms has resulted in a plethora of research

to establish these mechanisms and explore antimicrobial alternatives (Barton, 2014; Francino, 2016; Langdon et al., 2016; Iizumi et al., 2017; Zeineldin et al., 2019). While antibiotics are typically administered against acute infections, many are not specific for pathogenic microorganisms and thus drastically alter the gut microbiome structure and composition post antibiotic treatment (Langdon et al., 2016). For example, shifts in the microbiome after antibiotic usage may revert after cessation of treatment but some communities never regain their pre-treatment composition or structure which may lead to susceptibility for opportunistic pathogen colonization in the host (Jernberg et al., 2010; Pettigrew et al., 2012; Zeineldin et al., 2019). Initiation of a more sustainable production-focused mindset in the pork industry has preceded an investigation into a shift away from the use of antibiotics in commercial animal agriculture (Zeineldin et al., 2019). Due to its widespread impact on health, immune function, and metabolism, impacting the gut microbiome via probiotics, prebiotics, and postbiotics remains one of the most promising possibilities for replacing antibiotics (Reid and Friendship, 2002; Yang et al., 2015). Variability in application usually regarding dosing level or specific strain usage, can result in the effects of probiotics being somewhat confounding; however, several reviews reported general improvement of multiple growth performance parameters after analyzation of many studies utilizing probiotics (Liao and Nyachoti, 2017; Liu et al., 2018). Thus, while antibiotics may provide short term solutions for improvement of swine production, finding alternative and more sustainable methods for preventing and treating disease and improving swine growth and health through probiotics, prebiotics, and postbiotics will prove quintessential for the pork production industry.

1.6 Swine Reproduction

1.6.1 Sow Health and Reproductive Performance

As discussed briefly above, sow reproductive efficiency is the basis for all pig production and selection for highly prolific animals has produced a number of negative outcomes on piglet health, growth, and development as well as sow health (Knol et al., 2002; Quesnel et al., 2008; Kim et al., 2013; Tokach et al., 2019; Foxcroft et al.). Pigs weaned per sow per year as a measure of sow productivity is not an adequate measurement for sow quality, piglet quality, or piglet and sow welfare (Koketsu et al., 2017). There are many factors which contribute to sow productivity including housing, age, and genetics; however, nutrition and health constitute major controllable factors influencing level of sow productivity, longevity, and reproductive performance (Allan and Bilkei, 2005; Shen et al., 2011; Koketsu et al., 2017; Koketsu and Iida, 2017; Costa et al., 2019). Drastically increased litter sizes means sows must respond and adjust their average daily feed intake, or nutrient intake, accordingly in order to support the larger litter during gestation and lactation (Kim et al., 2013). Traditional sow diets likely underfeed nutrients vital for sow health and productivity which induces a catabolic state in those animals struggling to meet increased nutritional demands of gestation or lactation (Kim et al., 2013). Sows exhibiting a catabolic state show increased production of reactive oxygen species, an important indicator of sow health, and increases in ROS expression leads to inferior reproductive performance and decreased ability for a sow to nurture a litter (Flowers and Day, 1990; Berchieri-Ronchi et al., 2011; Kim et al., 2013). As sow health and reproductive performance are tightly intertwined, the relationship between sow diet, microbiome, oxidative stress, and sow productivity have been investigated (Allan and Bilkei, 2005; Wang et al., 2018; Costa et al., 2019; Wang et al., 2019). Composition of

the sow gut microbiome has been shown to change due to oxidative stress or with changes in the health status of the sow (Shao et al., 2020). Different gut microbiome compositional changes have been correlated with productivity and health in sows; for example, increases in *Bacteroides* and SCFA-producing bacteria and decreases in microbial diversity are associated with higher producing, healthier sows (Callens et al., 2015; Shao et al., 2020; Uryu et al., 2020; Xu et al., 2020). In addition, high dietary fiber inclusion in sow diets, has many beneficial prebiotic effects including increased production of SCFA, reduced concentration of pathogenic bacteria in the gut, and reduced digesta passage rate (Oliviero et al., 2009; Agyekum and Nyachoti, 2017; Jiang et al., 2019; Wu et al., 2020). Furthermore, increasing dietary fiber has been associated with improved farrowing performance and reduced opportunistic pathogens in pregnant sows (Monteiro et al., 2022). The wide range of mechanisms by which a sow's microbial community may be influenced by dietary nutrients and subsequent interaction with reproductive performance has been reviewed (Veum et al., 1995; Kim et al., 2013). Sow diet, microbiome, health, and reproductive productivity are highly connected and finding ways to shift the sow's microbiome to improve health is essential for improved reproductive efficiency. Several studies have investigated the impact of yeast culture supplementation on sow reproductive performance with mixed results as reproductive performance is not always influenced but litter weight gain can be improved potentially via milk production (Kim et al., 2008; Kim et al., 2013; Yuan et al., 2015). While the effect on the sow may be confounding, these results indicate that sow health and offspring are likely intertwined via sow milk or other mechanisms.

1.6.2 Sow Health and Offspring Health

A sow's health and nutritional status mediates health and development of offspring beginning in utero, throughout lactation, and may continue to have large impact on post-wean health and performance of subsequent progeny (Vinsky et al., 2006; Oliviero et al., 2019). Litter size has increased drastically in modern sows, this has led to longer farrowing duration inducing more stress on the sow, intrauterine growth restricted and low viability piglets, increased birth weight variation, and decreased colostrum intake per piglet (Rooke and Bland, 2002). Piglets are born with a functional and mature innate immune system; however, they lack inherent immunoglobulins and therefore must acquire maternal immunoglobulins via colostrum (Rooke and Bland, 2002). This is termed passive immunity and is vital for piglets' survival in the first 3-4 weeks of life, passive immunity is essential for newborn piglets as decreased colostrum intake in a piglet's first 24 hours has been associated with negative effects on piglet survival (Devillers et al., 2011; Quesnel et al., 2012). Colostrum composition and intake are critical for a piglet's health, survival, and growth in its first 24-36 hours as gut closure inhibits absorption of immunoglobulins from colostrum approximately 36 hours post-parturition (Rooke and Bland, 2002; Devillers et al., 2011). Failure of piglets to obtain colostrum is the primary cause for piglet mortality in the first days post-parturition and colostrum and milk intake have been shown to have a large impact on piglet gut and immune system development (Salmon et al., 2009; Graugnard et al., 2015). A piglet needs approximately 200-250 grams of colostrum to minimize mortality and maximize body weight gain (Salmon et al., 2009). As litter size increases, the demand for more immunoglobulin production in the sow to support colostrum production and sufficient passive immunity for more piglets is greatly increased; therefore, finding ways to

enhance immunoglobulin production in the sow is essential (Rooke and Bland, 2002). Dietary nutrients such as energy intake or essential fatty acids have been shown to affect milk and colostrum composition and yield (Rooke and Bland, 2002). Supplementing dietary probiotics or prebiotics to sows may have potential to beneficially impact colostrum composition via shifting maternal microbiome composition or stimulating the sow's immune system to produce greater amounts of immunoglobulins; hence, improving piglet health (Scharek et al., 2007; Jang et al., 2013; Zanello et al., 2013; Jarosz et al., 2022). Sow colostrum is characterized starting with elevated levels of IgG as IgG being the major absorbed immunoglobulin during the first 24 hours post-parturition and then being replaced with increasing levels of IgA concentration in milk to provide passive mucosal protection for the piglet (Rooke and Bland, 2002; Devillers et al., 2011). Supplementation of sow gestation and lactation diets with yeast increased IgG concentration in sow colostrum and piglet plasma (Kogan and Kocher, 2007; Scharek et al., 2007) which suggests that dietary yeast supplementation results in increased IgG in colostrum which is then transferred to progeny. Dietary yeasts may also prevent IgA concentration in sow milk from decreasing throughout lactation (Kogan and Kocher, 2007). Probiotic yeast supplementation likely stimulates the maternal immune system via β-glucan and mannan-oligosaccharides (MOS) on its cell wall as this mechanism is supported by studies reporting that supplementation of MOS increased concentration of immunoglobulins in sow milk (Jurgens et al., 1997; Kogan and Kocher, 2007; Scharek et al., 2007). Subsequent impact of dietary yeast supplementation to sows on piglet performance is unclear with some reporting no effect and others demonstrating improved performance of piglets; however, improving colostrum quality may provide a greater

impact on piglet survivability than piglet performance (Kogan and Kocher, 2007; Scharek et al., 2007; Shen et al., 2017; Rocha et al., 2022). The effect of yeast supplementation appears to improve progeny immune response post-wean, however; the impact on sows and their offspring on post-weaning performance is unclear (Shen et al., 2009a; Shen et al., 2011; Nowland et al., 2019c; Rocha et al., 2022).

1.6.3 Microbial Succession

While piglets receive passive immunity via colostrum post-parturition, their microbiome is largely colonized during the process of parturition when the fetus travels from a sterile environment inside the sow to a microbially diverse environment (Nowland et al., 2019c). There is some dispute as to whether there is bacteria present in amniotic fluid which suggests some in utero microbial colonization of piglets; however, this remains unknown in livestock species but if colonization happens in utero, it is likely dependent on placentation structure (Nowland et al., 2019c). Human neonates delivered via Cesarean section possess altered microbial populations compared to those delivered vaginally and Cesarean section neonates have been suggested to have increased incidences of health conditions which underlines the importance of microorganisms harbored at birth (Dominguez-Bello et al., 2010; Yang et al., 2016; Nowland et al., 2019c). In neonatal piglets the gut microbial community is essential for several protective, metabolic, and trophic roles including acting as a barrier against pathogens, aiding digestion and metabolism of colostrum and milk, breaking down toxins and drugs, synthesizing vitamins, absorbing ions, and supporting growth and differentiation of the intestinal epithelium (Yang et al., 2016; Nowland et al., 2019c). Colostrum composition, milk quality, and the environment neonatal piglets are born into are likely the major

factors impacting initial microbial colonization (Nowland et al., 2019c). Maternal milk contains bacteria and other factors which are instrumental in establishing a balanced, healthy intestinal microbiome presumably due to the importance of the enteromammary axis (Gomez-Gallego et al., 2016; Morissette et al., 2018; Nowland et al., 2019c). Immediately after birth the gastrointestinal tract of piglets is colonized by aerobic bacteria which increase until approximately 7 days post birth then are largely replaced by anaerobes and coliforms (Swords et al., 1993; Knecht et al., 2020). The specific colonization of piglets vary to some extent by study; however, bacteria in the *Streptococcaceae* family, *E. coli, Shigella flexneri,* and some *Lactobacillus* species dominate in the first 2-3 days post-parturition with a secondary colonization occurring around day 3 so the piglet microbiome is dominated by *Lactobacillacea* and *Clostridiaceae* species (Swords et al., 1993; Konstantinov et al., 2006; Petri et al., 2010; Knecht et al., 2020). The presence of facultative aerobic or anaerobic bacteria is concurrent with colostrum intake and then a shift to *Lactobacilli* and *Bifidobacterium* follows since milk contains these lactic acid bacteria (Knecht et al., 2020). Throughout lactation, higher weight gain piglets have increased populations of *Bacteroidetes, Bacteroides,* and *Ruminoccocaceae* species and lower populations of *Actinobacillus porcinus* and *Lactobacillus amylovorus* species than low weight gain piglets (Morissette et al., 2018). These observed differences in growth performance and microbiome in pre-wean piglets indicate that colostrum and milk intake and composition may impact long-term growth via the gut microbiome (Knecht et al., 2020).

Upon parturition, piglets are conceived into a microbially diverse environment where they are exposed to the sow's feces, skin, and mucosal surfaces and therefore the piglet's

microbiome is likely largely dependent upon the sow (Nowland et al., 2019c). Indeed, research shows that piglets raised in a commercial setting possess a more diverse gut microbiome than piglets raised in isolators on milk formula and that this difference in microbial community influenced piglet immunological development (Inman et al., 2010). Likewise other research indicates that the piglet gut microbiome composition is similar to bacteria found on environmental surfaces such as the floor or the sow's nipple and becomes more similar to the sow fecal microbiota as lactation progresses (Chen et al., 2018). Sow nutrition can likely influence progeny microbial communities in early life presumably via milk composition and metabolites and sow fecal microbial composition (Ma et al., 2020; Liu et al., 2021). The opportunity to manipulate the microbiome in early life provides an avenue for influencing appropriate microbial colonization and immune development given the criticalness that appropriate microbial colonization and immune development have during the pre-weaning period (Cahenzli et al., 2013; le Doare et al., 2018; Nowland et al., 2019c). The impact of sow nutrition, parity, farrowing crate hygiene, sow skin and udder hygiene, piglet fostering, iron injections, and age of weaning on microbial succession remains relatively unclear highlighting the need for more research in this area (Nowland et al., 2019c). After an introduction to solid feed and weaning, the influence of the sow on piglet microbial communities diminishes as there is a shift to more abundant fibrolytic and butyrate producing bacteria such as *Ruminococcus*, *Lachnospira*, *Roseburia*, *Eubacterium*, and *Prevotella* (Bian et al., 2016; Choudhury et al., 2021). Individual species of bacteria have been identified to have an impact on the health and growth of piglets pre-wean and post-wean and these known species have been reviewed (Nowland et al., 2022).

1.7 Post-Wean

1.7.1 Piglet post-wean performance

After weaning, piglets enter into the greatest growth phase of their life. They are provided ad libitum feed and water in order to maximize growth performance and efficiency. Many strategies are employed to maintain piglet feed intake as modern genetics have greatly increased feed efficiency but have simultaneously decreased voluntary feed intake (Webb, 1989). Feed intake represents a direct means to influence growth rate, feed efficiency, and carcass quality in swine and therefore has a great impact on profitability (Nyachoti et al., 2004). Feed intake and growth is governed by many factors including thermal, social, and physical environment, health, genotype, and diet which has been reviewed (Nyachoti et al., 2004). Most important to this review, regarding influencing the microbiome, are the health, age, and physiological status of the animal and the diet. Decreased health status is related to decreased feed intake and reduced growth performance as energy is shifted from lean deposition to immune responses (Nyachoti et al., 2004). A pig's age and physiological status impact its gut microbial community (Nyachoti et al., 2004). Age and physiological status also impact the pig's capacity to ingest, digest, and metabolize dietary nutrients as evidenced by increased daily feed intake to meet daily nutrient requirements as body weight increases (Nyachoti et al., 2004). Dietary factors also impact voluntary feed intake such as feed bulk, diet nutrient content and balance, feed additives, dietary contaminants, water availability, and feed presentation (Nyachoti et al., 2004). In addition to the impact of the microbiome on health discussed above, the microbiome is influenced by the diet and the microbiome can directly influence diet digestibility (Lee et al., 2014; Frese et al., 2015).

1.7.2 Post-wean microbiome and growth

Specific microbial species populating the gut microbiome of piglets post-wean has been discussed above; however, their impact on nutrient digestion and utilization and health remains to be discussed. Diversity and richness of the microbiome increases with age of the pig and this increase in diversity and richness indicates a fully developed swine gut microbiome pre-marketing (Lu et al., 2018; Wang et al., 2019). Interestingly, post-wean changes in gut microbiome composition do not appear to happen suddenly but seem to take 7 to 9 days to adapt to a new diet and subsequent gut physiological changes (Wang et al., 2019). Longitudinal studies in growing pigs up to market weight possess great promise in identifying species of beneficial microbes and elucidating the mechanism by which they elicit an effect on the host (Kim et al., 2011; Lu et al., 2018; Wang et al., 2019). *Prevotella* species dominate the gut microbiome for many of the solid feed phases in growing pigs as members of *Prevotella* are associated with plant food-based diet and fiber digestion (Wang et al., 2019). Diet is the major determinant of the swine gut microbiome with neutral detergent fiber (NDF) from corn and soybean meal having presumably the greatest individual impact on the microbial composition of the gut based on the diets and data (Wang et al., 2019). *Prevotella*-enriched groups of animals may represent individuals consuming plant polysaccharide rich diets while an *Escherichia*-enriched community in the presence of *Enterococcus* may signal gut health dysbiosis (Lu et al., 2018). Improving feed efficiency or growth by identifying probiotic gut microbes has become a novel strategy in the swine industry. Species of *Turicibacter* have been linked to improved immunomodulation and increased body weight, *Clostridium butyricum* has been linked to improved body weight likely through butyric acid

production and immunomodulation, species of *Clostridiaceae* have also been positively correlated with body weight, and *Streptococcus* species and *Lactobacillus mucosae* have been linked to growth and appear to be involved in intestinal permeability and barrier function (Wang et al., 2019). Supplementation with dietary probiotics has been shown by many studies to have a positive impact on post-wean growth performance of pigs and a potential impact on diet digestibility (Giang, 2010; Giang et al., 2011; Lee et al., 2014; Jørgensen et al., 2016).

1.8 Yeast Feed Additives

1.8.1 Yeast fermentation by-products

Ethanol production has been an essential piece of commercial United States corn production since the 1940's with a massive increase in production in the 1990's (Abebe, 2008). To increase efficiency and sustainability of the ethanol industry, co-products of ethanol fermentation began to be utilized as novel feed ingredients in commercial animal diets (Arora et al., 2010; Distillers grains and other valuable, 2021). Utilization of fermented ingredients has been recognized for their value in animal diets by providing enhanced digestibility, increased immune function, and improved performance of animals (Plumed-Ferrer and von Wright, 2009; Shurson, 2009; Keller et al., 2020).

1.9 Conclusion

Increased demands on the metabolism and health of the modern sow due to a significant increase in litter size has called for intervention methods that would assist in mitigating negative consequences of large litters. Use of various feed additives to manipulate the gut microbiome in sows in an attempt to reduce the manifestation of stress and increase

performance and health of her offspring has occurred. However, the effectiveness of each additive varies, and few studies have investigated the impact of modulating sow microbiome on piglet microbial succession, health, and growth. Little is known about how different sow gut microbial compositions translate via microbial succession to a piglet's gut microbiome during the suckling period and into the post-wean period. Characterizing specific sow gut communities which correlate to improved health or growth performance in their offspring would provide potential for a host of strategies to utilize microbial feed additives on a regular basis such as targeted probiotic supplementation.

1.10 Hypothesis and Research Objectives

Diamond V Mills works to produce high quality microbial fermentation products as animal postbiotic feed additives and research is needed to test the efficacy, safety, and performance of these novel products in swine (Diamond V Mills Inc.). Thus, a study was conducted to further elucidate the potential of a yeast postbiotic to mitigate the negative implications that arise with large litter size via the sow microbiome. The study objective was to observe the impacts of including a yeast fermentation postbiotic in gestation and lactation diets on sow reproductive performance, sow fecal microbiome composition, offspring performance through the nursery, and offspring fecal microbiome composition. It was hypothesized that the inclusion of the yeast postbiotic would influence the sow microbiome composition and offspring microbial communities and ultimately improve offspring performance during the suckling and the nursery period.

Chapter 2

2.0 Liquid postbiotic supplementation alleviated impact of low nutrient swine diets

2.1 Abstract

During warm summer months, dietary intake levels of finishing pigs can drastically decrease, which may impact overall growth performance through macronutrient insufficiencies resulting from lower intake. The goal of this 77-d finishing pig trial was to investigate the inclusion of Saccharomyces *cerevisiae* fermentation prototype (**SCFP**) in diets with reduced nutrient content on growth performance. A total of 44 pens (237 finishing gilts and barrows) were assigned to one of 4 dietary treatments: **CON** formulated in 4 diet phases using corn, soybean meal, and DDGS, **CON+** where 1% SCFP was added, and **RED5+** and **RED8+** where protein, amino acids, and energy were reduced 5% and 8%, respectively with the inclusion of soy hulls plus 1% SCFP. Dietary NDF was 16% and 17.5% in RED5+ and RED8+, respectively and 13% in CON and CON+. Pigs and feeders were weighed every 2 weeks; data was analyzed as a completely random design with pens as the experimental unit. In d28-d42, gain:feed ratio (G:F) was lower ($P < 0.05$) in RED8+ and RED5+ pigs than CON and CON+, and in d42-d56 G:F was lower ($P < 0.05$) in RED5+ than CON+ with CON and RED8+ intermediate. In d56-d70, average daily gain and BW were lower ($P < 0.05$) in RED8+ pigs than CON, and G:F was lower ($P < 0.05$) in RED8+ than CON and CON+. In all other weigh periods, BW was similar across all groups. There was a decreased digestibility of the RED5+ diet noted in Phase 2 diets compared to the CON diet. The similar growth and feed intake between CON, CON+, and RED5+ pigs suggest that the SCFP may provide a nutrient uplift by way of enhanced nutrient digestion to offset the 5% reduction in dietary protein and energy.

Keywords: finishing pig performance, Saccharomyces *cerevisiae* fermentation product

2.2 Introduction

Heat stress is well known to reduce feed intake and therefore performance of growing-finishing pigs (Xiong et al., 2020). Summer in the Midwest United States is often concurrent with ambient temperatures above the thermal neutral zone of swine which can lead to heat stress and reduced feed intake (Quiniou et al., 2000). The result of reducing feed intake in efficiently growing animals is decreased growth performance and therefore longer time to reach market weight during times of high temperatures. In a review, Elghandour et al. (2019) outlined the use of yeast strains in nonruminants and established the basis for its success as a probiotic (Elghandour et al., 2020). It has been shown that diet costs could be decreased, and performance increased during heat stress by using dietary prebiotics or probiotics in growing-finishing diets (Price et al., 2010).

Many different yeast products, including active dry yeast, yeast cell wall, and yeast culture, have been investigated for their effects on the immune system, growth performance, and gut microbiome of swine (Alugongo et al., 2017; Jurgens et al., 1997; Kornegay et al., 1995; van der Peet-Schwering et al., 2007). Active dry yeast was shown to improve offspring growth efficiency when supplemented to sows during gestation and lactation and their offspring during the growing period (Jurgens et al., 1997). Yeast culture contains live and dead yeast cells, culture media, and many metabolic products which include proteins, lipids, vitamins, and amino acids among other compounds and nutrients (Alugongo et al., 2017). *Saccharomyces cerevisiae* yeast culture has been shown to increase growth performance of weanling and growing pigs, most notably increasing their gain: feed ratio (G:F) (Kornegay et al., 1995; van der Peet-Schwering et

al., 2007). However, when yeast culture was supplemented to growing-finishing pigs along with a reduced protein diet, the yeast culture did not affect growth performance while the reduced protein decreased G:F (Bowman and Veum, 1973).

Increasing the palatability and digestibility of diets is one method of sustaining performance during periods of low feed intake such as during heat stress. There have been mixed results reported regarding the effect of yeast on diet dry matter digestibility with several reporting increased digestibility when supplementing yeast culture (Shen et al., 2009b; Shi and Kim, 2019). However, researchers showed that supplementing yeast culture had no effect on digestibility in early weaned pigs (Veum & Bowman, 1973). It was also observed that live yeast supplementation had no effect on diet digestibility (Li et al., 2006). Kornegay et al. (1995) demonstrated that yeast culture supplementation in weaned pigs maintained growth performance when fed a high-fiber diet suggesting increased fiber digestibility (Kornegay et al., 1995). Currently, information is scarce regarding the use of liquid yeast culture and the associated effects on growth performance and digestibility during later finishing phases as previous studies have mostly utilized weanling or weaned pigs (Veum and Bowman, 1973; Kornegay et al., 1995; Jieyun Li et al., 2006; van der Peet-Schwering et al., 2007; Shen et al., 2009b; Price et al., 2010; Shi and Kim, 2019). Furthermore, improving nutrient delivery and nutrient availability in swine diets is especially important during periods of low intake to maintain growth efficiency to remain economical (Boland et al., 1999). Therefore, the goal of this study was to investigate how supplementation with a liquid *S. cerevisiae* fermentation prototype (SCFP), a postbiotic, impacted growth performance and total tract nutrient digestibility in finishing pigs during summer in the Midwest United States when fed a

diet with high fiber and reduced protein and energy. It was hypothesized that SCFP would maintain performance in finishing pigs fed high fiber and reduced protein and energy diets through increased dietary nutrient digestibility.

2.3 Materials and Methods

This experiment was conducted at the South Dakota State University Swine Education and Research Facility, Brookings, SD. The South Dakota State University Institutional Care and Use committee approved the protocol (IACUC#2105-028E) used in this study.

2.3.1 Animals, management, and experimental design

A total of 237 gilts and barrows, Large white/Landrace x Duroc; 32.2 (3.6 kg) (58 [2 days] old), housed in 44 1.8m x 2.4m fully slatted concrete floor pens were allotted in a completely randomized design to one of four treatment groups (11 replicates/treatment; 4 - 6 pigs/pen). Pigs were housed by gender and had been part of a nursery trial evaluating the impact of medium chain fatty acid inclusion on pig performance. Previous experimental treatments were balanced across the treatments used in this study and allowed a 16-day washout period where all pigs were fed the same diet formulated to meet NRC (2012) nutrient requirements for the relevant stage of production for these animals. Each pen contained an individual dry feeder with 2 feeding slots and one cup waterer providing ad libitum access to feed and water. Pigs removed from the trial due to poor health, death, or euthanized were recorded with date and weight at removal. Feeders, waterers, and pigs were checked daily between 0600 and 0800h.

2.3.2 Dietary treatments

The four experimental treatments (Table 2.1) were a control diet (CON) based on corn, soybean meal, and DDGS, the control diet which included the SCFP supplemented at 1% (CON+) at the expense of corn, a diet with 5% less protein, amino acids, and energy and SCFP supplemented at 1% (RED5+), and a diet with 8% less protein, amino acids, and energy and SCFP supplemented at 1% (RED8+). The diets varied in fiber content with CON and CON+ averaging 13% NDF across the diet phases, RED5+ averaging 16% NDF, and RED8+ averaging 17.5%. Experimental diets were provided over four phases with phase 1, 2, and 3 representing 28, 28, and 14 days, respectively. Phase 4 began on day 70 (8 days before the first pigs were marketed from the room) and was continued until all pigs were marketed. All diets were formulated to meet or exceed NRC (2012) vitamin and mineral recommendations within phase, CON and CON+ met or exceeded energy, protein and essential amino acid recommendations within phase; essential amino acid to lysine ratio was held constant between diets.

2.3.3 Sampling and data collection

Diet samples were collected from every feeder 7 days after the start of a new phase and stored at -20 ºC until analysis. A pooled sample for each diet and phase was ground, placed into sealed bags and shipped for analysis of crude protein, crude fiber, ash, amino acid composition, and crude fat. After 14 days on each of diet phase 1, 2, and 3 grab floor fecal samples were collected and stored at -20 ºC until analysis.

At the start of the study and every 14 days from d0 through d70, individual pig body weight (BW) and feed disappearance was measured and average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F) was calculated on a pen basis. The ADG and ADFI were calculated based on “pig days” which are defined as the

number of pigs in a pen on each day multiplied by the respective number of days to account for pigs removed throughout the study. Market ready pigs were shipped in 3 loads with the first load on day 78 of the study and the final load on day 94. Prior to shipping, individual pigs were weighed for collection of individual body weight at market.

Fecal samples collected were freeze - dried prior to analysis. Prior to freeze - drying, a wet weight was obtained for each fecal sample. After each sample was determined to be completely dried, a dry weight was obtained for each fecal sample and percent dry matter (DM) was calculated. Individual fecal samples were ground using a 0.75 mm sieve. Digestibility of each diet was elucidated utilizing inherent acid insoluble ash (AIA) determined in duplicate for feces and in triplicate for diets (McCARTHY et al., 1974). The AIA was analyzed according to Coca-Sinova et al (2011) with modification (De Coca-Sinova et al., 2011). Briefly, fecal (3g) and feed (6g) were ashed at 500 ºC for 20 h and 24 h, respectively. Following ashing, samples were cooled in a desiccator then 5 and 10 mL of 4 N HCl were added to the fecal and feed sample tubes, respectively and placed in a heating block (131 ºC) for 2 h, then cooled and centrifuged at 1,773 x g for 10 min. The supernatant was removed, and the pellet washed twice with 5mL distilled water. Samples were centrifuge between each wash and after the final wash before drying overnight at 90 ºC. Dried samples were ashed for 5 - 8 h. Ashed samples were cooled in a desiccator and weighed. Gross energy (GE) in diets and feces was determined in duplicate using bomb calorimetry (Parr Instrument Company 6400 Calorimeter, city, state, USA).

2.3.4 Statistical Analysis

The UNIVARIATE procedure of SAS (SAS Inst., Inc., Cary, NC, USA) was used to confirm the homogeneity of variance and to analyze for outliers. Performance data were analyzed as a randomized complete block design using the PROC MIXED procedure in SAS. In the model, the main effects of dietary treatments were tested considering pen as experimental unit, gender as the blocking factor, and initial BW as a covariate for all dependent variables. Tukey's adjusted means test was used to detect differences between treatment groups where $P \leq 0.05$ is considered significant.

2.4 Results

In general pigs were healthy and there were few veterinary treatments during the entire experimental period. The temperature inside the housing facility was recorded daily throughout the experimental period (Figure 2.1). Average room temperature was at or above the age specific target temperature for all but 4 days during the entire experimental period. The analyzed nutrient composition of the diets were similar to formulated values with the energy and protein 5% lower in the RED5+ diet and 8% lower in the RED8+ diet compared to the control.

2.4.1 Growth Performance

There was no effect of SCFP on ADFI, ADG, or BW from d0 - 56 and from d70 - 77 (Table 2.2). However, from d28 - 42, G:F was lower (P = .017) in RED8+ and RED5+ than CON and CON+ pigs, and from d42 - 56 G:F was lower (P = .024) in RED5+ than CON+ with CON and RED8+ intermediate. From d56 - d70, ADG (P = .008) and BW (P = .017) were lower in RED8+ pigs than CON, and G:F was lower (P = .002) in RED8+ than CON and CON+. In all other weigh periods, BW was similar across all groups (P >

0.05). Assessing the entire experimental period (d0 - 77), a numerically lower G:F was observed for RED5+ than for CON+ with CON and RED8+ intermediate; however, no statistical differences were noted. Throughout the experimental period, average room temperature rose and remained consistently above daily setpoint (Figure 2.2).

2.4.2 Nutrient Digestibility

In phase 1 and phase 3 there were no differences in GE digestibility and total tract DM digestibility for any of the diets (Table 2.3). In phase 2, both GE digestibility and total tract DM digestibility were significantly lower in the RED5+ diet than the CON diet ($P < 0.05$) with the CON+ and RED8+ diets intermediate.

2.5 Discussion and conclusion

The objective of this study was to investigate how supplementation with a liquid SCFP impacted growth performance and total tract nutrient digestibility in finishing pigs during summer in the Midwest United States when fed a diet with high fiber and reduced protein and energy. Heat stress in growing - finishing pigs has been well studied for its impact on growth performance and specifically its effect on feed intake. Quiniou, Dubois, and Noblet reported that temperatures between 19 and 29 ºC decreased voluntary feed intake in swine by 48 - 77 g/d/ºC, and attributed this decrease to limited gut capacity or gut fill (Quiniou et al., 2000). In the experimental period, the average daily temperature fluctuated but remained consistently between 21 to 29ºC. Addition of soy hulls without adding fat was used as a means to reduce energy and protein concentrations in the diets

which also increased dietary fiber, particularly NDF content (Mauch et al., 2018). Typically, a pig will increase volume of feed consumed to meet energy requirements for performance when provided a reduced energy diet (Schinckel et al., 2012). This increase in intake contributes to gut fill and compounds the issue of maintaining voluntary feed intake in swine during periods of high temperature. Although there were slight numerical differences at the beginning of the experimental period, there were no significant differences in ADFI at any point throughout the trial. Addition of SCFP may contribute to maintaining voluntary feed intake during periods of high environmental temperatures in growing-finishing pigs to retain performance.

The lack of difference in growth among treatments demonstrated that supplementation with SCFP can provide a nutrient uplift to finishing pigs fed diets with high fiber and reduced protein and energy during extended periods of elevated temperatures. Similar conclusions regarding growth performance were observed by Kornegay et al. (1995) in weanling pigs fed a high fiber diet supplemented with yeast culture; however, performance was not improved with weanling pigs fed whey supplemented with yeast culture (Kornegay et al., 1995). Bowman and Veum (1973) did not observe a difference in performance of pigs from 11 kg -100 kg fed diets containing 16% or 18% protein when supplemented with yeast culture (Bowman & Veum, 1973). In several studies, growth performance in weanling pigs was improved with supplementation of yeast culture when compared to a standard diet (Dávila-Ramírez et al., 2020; van der Peet-Schwering et al., 2007). In the study, SCFP did not appear to provide nutritional value above what the standard finishing diet provided; however, was able to normalize performance in a reduced energy and protein diet to only marginal

numerical differences between pigs fed a reduced diet compared to pigs fed a standard corn/SBM finishing diet over the entire experimental period. Overall, these sources and the study indicate that the effect of postbiotic supplementation using SCFP appears to be variable when supplementing in a complete diet but provides compensation for dietary nutrient deficiencies.

Addition of soy hulls to achieve the reduced diets used in the experiment notably increased their respective NDF (RED5+ had 3% more NDF than control and RED8+ had 5% more than control). In previous work Le Goff and Noblet (2001) and Le Gall et al. (2009) established that an increase of 1% dietary NDF results in a 0.8% reduction in energy digestibility which would equate to a 2.4% reduction in energy digestibility for the RED5+ diet and a 3.2% reduction in energy digestibility for the RED8+ diet (Le Goff and Noblet, 2001; Le Gall et al., 2009). In the experimental diets, the GE digestibility was lower in the reduced diets only in phase 2 while being similar across all diets in Phases 1 and 3. This data would seem to suggest that the addition of SCFP increased diet digestibility in high fiber diets to a level comparable with a standard corn/SBM finishing diet. Interestingly the decrease in GE digestibility during phase 2 was also shown in a decrease in DM digestibility and coincided with a decrease in G:F of pigs fed the reduced diets and with a spike in environmental temperature. However, the digestibility and G:F appeared to recover through parts of phase 3 in spite of sustained elevated temperatures.

Further analysis of growth performance in the experiment was run to estimate BW based on average daily lysine intake for each treatment group using an equation established by Loughmiller et al. (1998), as reported in NRC (2012), where 17.6 grams lysine are required for each kg gain in finishing pigs from 91-113 kilograms

(Loughmiller et al., 1998). The expected bodyweight based on the pigs average daily intake of lysine at day 77 for CON, RED5+, and RED8+ diets were 104.7 kg, 100.9 kg, and 97.02 kg respectively. Observed bodyweight in the experiment for CON, RED5+, and RED8+ diets at day 77 were 109.8 kg, 106.9 kg, and 105.9 kg respectively. All observed values for bodyweight were higher than the expected calculated values with CON pigs being 4% heavier, RED5+ pigs being 5.6% heavier, and RED8+ pigs being 8.4% heavier. This calculation supports the conclusion that supplementation with SCFP resulted in a nutrient uplift in diets with reduced energy and protein and thus has the potential to improve performance of pigs in the presence of lower energy and protein diets.

In conclusion, SCFP may improve body weight gain of finishing pigs fed a low energy and protein diet by 1-3% through enhancement of diet digestibility and maintaining feed intake.

Table 2.1. Diet formulation and nutrient composition of grower/finisher diets supplemented with *Saccharomyces cerevisiae* fermentation product and/or reduced crude protein and energy.

	Phase 1			Phase 2			Phase 3			Phase 4		
Ingredient (% inclusion)	CON[1]	RED5+	RED8+	CON[1]	RED5+	RED8+	CON[1]	RED5+	RED8+	CON[1]	RED5+	RED8+
Corn	64.78	58.89	55.52	66.63	59.46	58.46	67.70	63.98	61.02	78.88	73.19	64.22
Soybean meal, 46.5%	13.00	11.50	10.50	11.00	11.00	10.00	8.50	8.00	6.50	5.50	4.00	4.50
DDGS <6-9% oil	10.00	9.00	8.00	11.00	9.00	5.00	13.00	8.00	6.50	5.50	3.50	3.50
Soyhulls	8.00	16.50	22.00	8.00	17.00	23.00	8.00	17.00	23.00	7.50	16.50	25.00
L-Lysine HCl	0.55	0.52	0.47	0.44	0.37	0.35	0.36	0.32	0.32	0.33	0.33	0.33
L-Threonine	0.19	0.17	0.16	0.13	0.11	0.12	0.09	0.09	0.10	0.10	0.11	0.12
DL-Methionine	0.12	0.11	0.10	0.05	0.04	0.05	-	0.02	0.02	-	0.01	0.04
L-Tryptophan	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04
L-Valine	0.06	0.06	0.05	-	-	-	-	-	-	-	-	0.01
Soybean oil	0.80	-	-	0.70	-	-	0.70	-	-	0.70	-	-
Monocalcium phosphate	0.70	0.70	0.70	0.70	0.68	0.68	0.26	0.35	0.40	0.20	0.15	0.20
Limestone	1.30	1.05	1.00	1.00	0.95	0.95	1.00	0.85	0.75	0.90	0.82	0.68
Salt	0.25	0.25	0.25	0.11	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Grower Vitamin premix[2]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[3]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
SCFP[1]	-	1.00	1.00	-	1.00	1.00	-	1.00	1.00	-	1.00	1.00
Phytase[4]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Formulated												
ME, kcal/kg	3300.0	3137.0	3054.0	3300.0	3134.0	3042.0	3300.0	3134.0	3042.0	3300.0	3132.0	3014.0
SID Lys, %	0.98	0.93	0.88	0.85	0.81	0.76	0.73	0.69	0.66	0.61	0.58	0.61
Analyzed[5], %, as fed basis												
Dry matter	88.23	87.66	88.05	88.36	87.90	88.23	88.10	88.02	87.94	87.01	87.89	87.81
Crude Protein	15.43	14.62	13.95	14.53	14.54	12.74	12.63	12.91	11.82	10.74	10.12	10.66
Crude Fat	3.42	2.53	2.61	3.59	2.52	2.10	2.25	2.29	2.39	2.46	3.26	2.47
Lysine	1.16	1.15	1.13	1.04	1.03	0.94	0.91	0.96	0.91	0.68	0.70	0.73
Threonine	0.68	0.66	0.64	0.61	0.59	0.56	0.54	0.54	0.54	0.48	0.44	0.48

[1]*Saccharomyces cerevisiae* fermentation product (SCFP) added at 1% at the expense of corn to create the CON+ diet. (Diamond V Mills Inc., IA, USA)
[2]J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Calcium 55 mg, Vitamin A 11,000 IU, Vitamin D3 1,650 IU, Vitamin E 55 IU; Vitamin B12 0.044 mg, Menadione 4.4 mg, Biotin 0.165 mg, Folic Acid 1.1 mg, Niacin 55 mg, d-Pantothenic Acid 60.5 mg, Vitamin B16 3.3 mg, Riboflavin mg, 9.9 Thiamine 3.3 mg.
[3] J & R Distributing Inc. 518 Main Ave, Lake Norden, SD 57248 - USA. Minimum provided per kg of diet: Copper 16.5 ppm, Manganese 44.1 ppm, Selenium 0.03 ppm, Zinc 165 ppm.
[4]Quantum Blue phytase (AB Vista; Plantation, FL) supplying 500 phytase units/kg.
[5] Analyzed at University of Missouri Chemical Laboratories (University of Missouri, Columbia MO)

Table 2.2. Growth performance of growing-finishing pigs provided diets with or without liquid *Saccharomyces cerevisiae* fermentation product and/or reduced dietary protein and energy[1]

	Dietary treatments					
Item	CON	CON+	RED5+	RED8+	SEM	*P*-value[2]
Initial BW, kg	32.1	32.5	32.1	32.1	1.14	0.992
Phase 1, d0 - 14						
BW d14, kg	46.0	45.3	44.8	45.2	0.367	0.156
ADG, kg	0.97	0.93	0.90	0.93	0.028	0.357
ADFI, kg	2.25	2.13	2.10	2.18	0.078	0.594
G:F	0.4	0.44	0.43	0.43	0.017	0.950
Phase 1, d14 - 28						
BW d28, kg	59.0	58.4	58.4	58.4	0.476	0.751
ADG, kg	0.93	0.94	0.94	0.94	0.025	0.989
ADFI, kg	2.35	2.23	2.43	2.29	0.089	0.530
G:F	0.41	0.42	0.41	0.42	0.023	0.967
Phase 2, d28 – d42						
BW d42, kg	73.7	72.5	72.8	71.7	0.572	0.134
ADG, kg	1.01	1.01	1.01	0.95	0.023	0.202
ADFI, kg	2.51	2.51	2.65	2.53	0.041	0.053
G:F	0.40[a]	0.40[a]	0.38[b]	0.38[b]	0.047	0.017
Phase 2, d42 – d56						
BW d56, kg	88.4	87.2	86.9	86.4	0.681	0.233
ADG, kg	1.03	1.05	1.01	1.05	0.020	0.438
ADFI, kg	2.81	2.82	2.92	2.97	0.053	0.118
G:F	0.37[ab]	0.37[a]	0.35[b]	0.35[ab]	0.018	0.024
Phase 3, d56 - 70						
BW d70, kg	102.8[a]	101.1[ab]	100.1[ab]	98.1[b]	0.999	0.017
ADG, kg	1.01[a]	0.99[a]	0.95[ab]	0.84[b]	0.036	0.008
ADFI, kg	3.10	2.97	3.14	3.02	0.059	0.179
G:F	0.33[a]	0.33[a]	0.30[ab]	0.28[b]	0.018	0.002
Phase 4, d70 - 77						
BW d77, kg	109.8	107.7	106.9	105.9	1.23	0.153
BWpred[3] d93, kg	125.8	122.7	122.5	123.7	2.11	0.669
ADG, kg	0.99	0.94	0.97	1.11	0.072	0.373
ADFI, kg	3.28	3.10	3.33	3.36	0.080	0.100
G:F	0.30	0.30	0.29	0.33	0.019	0.464
Overall, d0 - 77						
ADG, kg	0.99	0.98	0.97	0.97	0.021	0.838
ADFI, kg	2.74	2.64	2.78	2.72	0.044	0.165
G:F	0.36	0.37	0.35	0.36	0.006	0.110

[1]Dietary treatments: CON, control; CON+, control plus 1% *Saccharomyces cerevisiae* fermentate prototype (Diamond V Mills Inc., IA, USA); RED5+, 5% lower energy and crude protein plus 1% fermentate prototype; RED10+, 10% lower energy and crude protein plus 1% fermentate prototype.
[2]abc – Letters indicate significant differences at $P \leq 0.05$ using Tukey's means separation test.
[3]predicted BW at d93 based on individual pig gain day 70 – 77. First cut of pigs removed at d78, second cut at d85, room emptied at d94.

Table 2.3. Nutrient digestibility of growing-finishing pigs provided diets with or without liquid fermentate and/or reduced dietary protein and energy[1]

	Dietary treatments					
Item	CON	CON+	RED5+	RED8+	SEM	*P*-value[2]
Dry matter digestibility, %						
Phase 1	94.26	93.49	93.99	93.87	0.395	0.588
Phase 2	94.46[a]	93.08[ab]	90.97[b]	92.48[ab]	0.751	0.020
Phase 3	95.84	95.49	95.58	95.79	0.204	0.567
Gross energy digestibility, %						
Phase 1	94.26	93.50	94.00	93.89	0.396	0.593
Phase 2	94.48[a]	93.04[ab]	91.68[b]	92.42[ab]	0.659	0.038
Phase 3	95.83	95.49	95.59	95.78	0.205	0.604

[1]Dietary treatments: CON, control; CON+, control plus 1% *Saccharomyces cerevisiae* fermentate prototype (Diamond V Mills Inc., IA, USA); RED5+, 5% lower energy and crude protein plus 1% fermentate prototype; RED10+, 10% lower energy and crude protein plus 1% fermentate prototype.

[2]abc – Letters indicate significant differences at $P \leq 0.05$ using Tukey's means separation test.

Figure 2.1. Daily room temperatures (average, high and low) throughout the experimental period
Blue line represents daily average temperature; Orange line represents daily low temperature; Grey line represents daily high temperature; Yellow line represents age specific temperature setpoint.

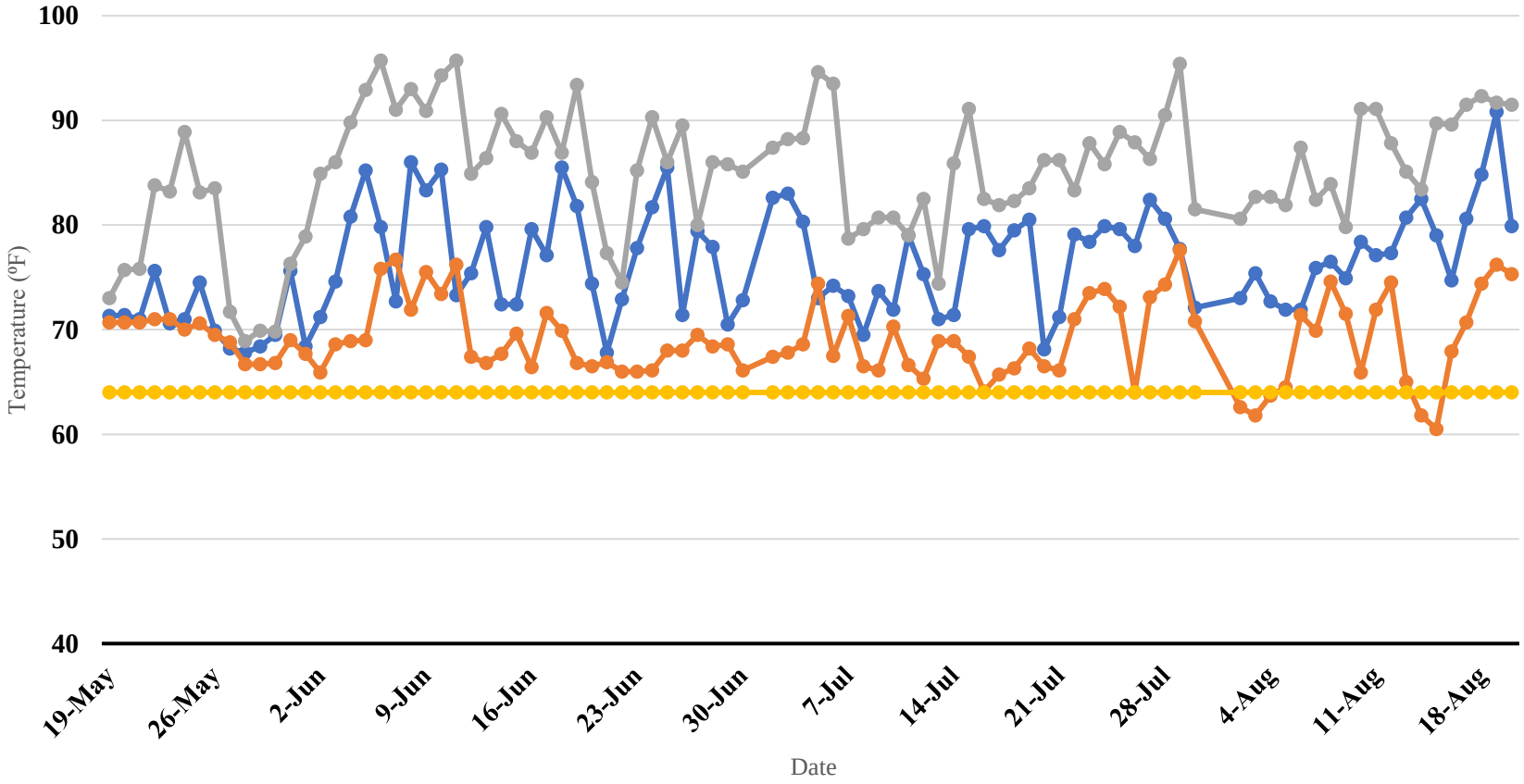
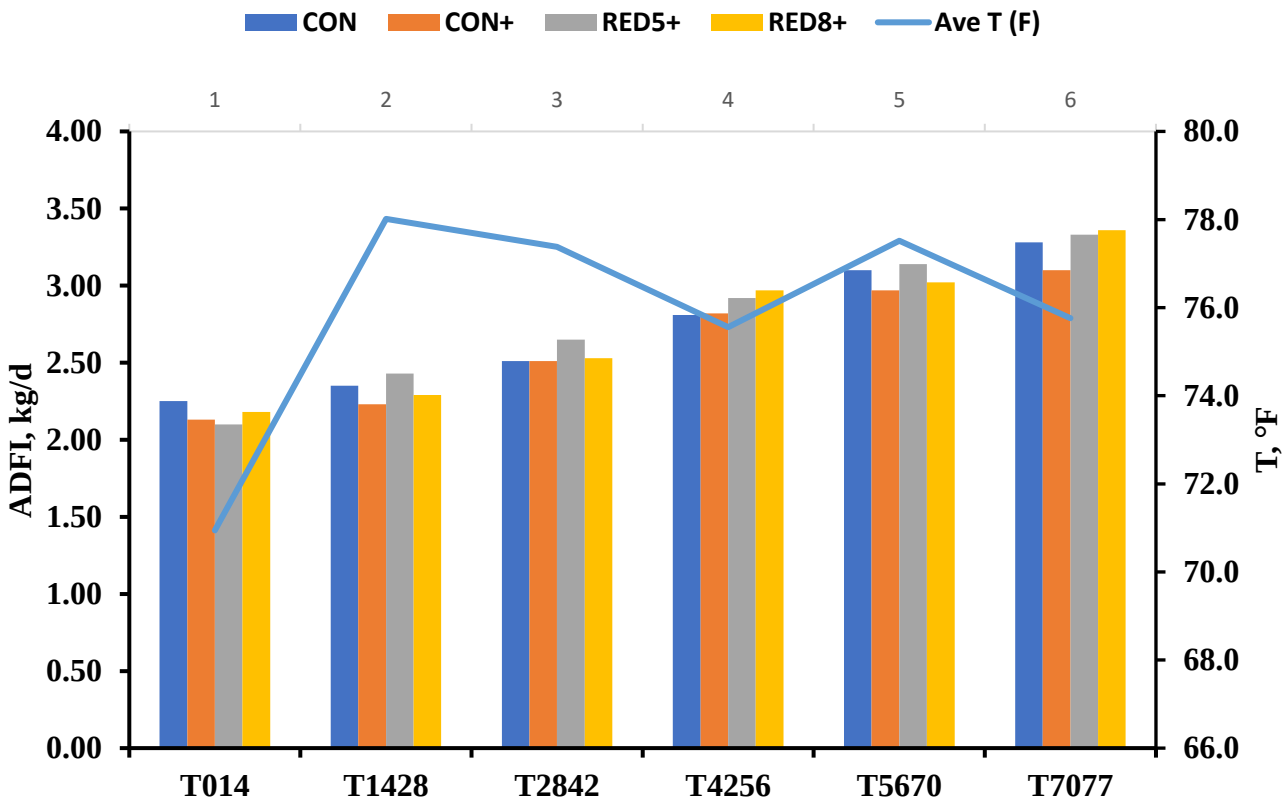


Figure 2.2. ADFI and average daily temperature during trial



Chapter 3

3.0 Yeast postbiotics to enhance reproductive performance of sows, sow fecal bacterial communities, nursery growth performance of offspring, and piglet microbial succession.

3.1 Abstract

Litter size and the resulting nutritional demand on the sow continue to increase while sow mortality and culling rate are also increasing. Non-nutritive feed additives may enhance sow health and thereby improve offspring growth and productivity after weaning. Development of the gut microbiome in piglets via microbial succession is critical for maximizing their productivity and providing stability for overcoming weaning stress. The objective of this study was to evaluate the impact of yeast-based postbiotic supplementation in gestation and lactation diets on offspring performance through the nursery period and on whether a yeast postbiotic could impact the sow fecal microbiome as well as affect microbial succession in piglets. Fifty-three gestating sows (parity 0 to 5; BW=242.7 ± 7.1 kg) in 2 breeding groups were blocked by parity and assigned to either a control (CON) diet or a diet supplemented with a yeast-based postbiotic (SUP) at 0.5% in gestation from d80 to 113 of gestation and 0.2% in lactation (d114 of gestation to weaning at 20 ± 2 d). Sow reproductive performance and offspring growth from birth to 65 d of age were monitored. At weaning, pigs were allotted to pens within maternal dietary treatment (10 pigs/pen; 31 to 32 pens/maternal treatment; 630 total pigs; BW=6.18 ± 0.86 kg) and all piglets received common nursery diets in a 4-phase program. Pigs were weighed at week 1, 2, 4, and 6 after weaning. Fecal bacterial composition was determined for 12 sows/treatment at d85 gestation, d1 lactation, and weaning and 1 piglet/sow at weaning and d7, 14, and 28 post-wean using Illumina MiSeq 2X300 sequencing of PCR-amplicons generated from the V1-V3 regions of the 16S rRNA gene.

A comparative analysis of the most highly represented Operational Taxonomic Units (OTU) was performed using the non-parametric Kruskal-Wallis sum-rank test and Wilcoxon pairwise test. Sow body weight and reproductive performance (piglets born alive/litter, 14.4 vs 14.1; piglet birth weight, 1.45 vs 1.48 kg; piglets weaned/litter, 13.0 vs 12.9; lactation sow feed intake, 6.4 vs 6.8 kg/d) was similar in CON and SUP sows, respectively. In the first week after weaning, pigs from SUP sows had a reduced tendency to lose weight (5.6 vs 11.0%). The numerically improved feed intake in the first week after weaning may explain the lower fallback rate in pigs from SUP sows. Across both sow groups, by 65 d of age, body weight (21.53 vs 21.76 kg), average daily gain (0.36 vs 0.37 kg/day), average daily feed intake (0.54 vs 0.53 kg/day), gain efficiency (0.67 vs 0.69 kg), and mortality (1.26 vs 1.60%) was similar in piglets from CON and SUP sows, respectively. In the initial fecal microbiome comparative analysis, no significant differences between sows which received CON or SUP diets or piglets were observed ($P > 0.05$), although, fluctuations in the abundance of specific OTUs were found over time in both sows and piglets. For instance, the abundance of OTU JK_30-00008, predicted to be a strain of *Lactobacillus amylovorous*, was elevated in sows at d85 (CON: 9.01%; SUP: 12.04%), dramatically reduced at d1 of lactation (CON: 1.00%; SUP: 3.03%), then recovered by weaning (CON: 9.41%; SUP: 9.74%). In contrast, the abundance of OTU JK_16-00021, predicted to be an uncultured *Peptostreptococcaceae*, remained elevated in sow fecal samples from both treatment groups at d85, d1 lactation, and weaning (CON: 10.6%, 15.05%, and 15.61%; SUP: 8.98%, 13.65%, and 14.47% respectively). In piglet fecal samples, the most abundant OTUs at weaning, d7, d14, and d28 were: JK_45-00042 (CON: 27.26%; SUP: 20.05%; no affiliation to any currently defined phylum), JK_137-

00038 (CON: 11.04%; SUP: 5.76%; unclassified *Yersiniaceae*), JK_30-00008 (CON: 13.64%; SUP: 14.11%; *Lactobacillus amylovorous*), and JK_51-00117 (CON: 7.66%; SUP: 5.32%; *Prevotella copri*), respectively. In piglets, the number of OTUs representing 50% of total sequence relative abundance increased with time (n = 5 OTUs at weaning, n = 18 at d7, n = 17 at d14, and n = 43 at d28) suggesting an increase in diversity with age. Yeast postbiotic in sow diet had limited impact on relative proportions of sow fecal microbiome and offspring microbial succession after weaning with greater piglet diversity expected due to dietary changes. In addition, several of the OTUs in greatest relative abundance in piglets, including JK_45-00042, JK_137-00038, JK_-42, and JK_-49 did not correspond to valid bacterial species. Together, these results underscore the need to identify prevalent unknown bacterial species in microbial community compositional shifts in the period around weaning.

Keywords: fecal microbiome, nursery pig performance, sow, microbial succession, yeast-based postbiotic

3.2 Introduction

Modern pork production in the United States has seen a drastic increase in prolificacy of the sow. The improvement in number of pigs born per sow has placed an ever increasing metabolic and nutritional demand on the sow to support a greater number of highly efficient progeny (Kim et al., 2013; Tokach et al., 2019). It is estimated that a sow must remain in the herd for at least 3 parities in order to provide a positive economic return; however, it is estimated that 40-50% of sows are culled annually (Rodriguez-Zas et al., 2003; Serenius & Stalder, 2004). Approximately one third of these culls are associated with reproductive problems and over half of these culls due to reproductive problems are

associated with first parity sows (Rodriguez-Zas et al., 2003; Engblom et al., 2008). This inadequacy in sow longevity is a reflection of the metabolic and physiological demands correlated with gestating and suckling a large number of offspring (Engblom et al., 2008; Kim et al., 2013; Tokach et al., 2019). In addition, the increases in litter size in the last 30 years have also resulted in an increase in piglet mortality, lower piglet birth weights, and greater within-litter variation which also reflects the inability of the sow to adequately support large numbers of offspring in her current metabolic status and nutritional provision (Knol et al., 2002; Quesnel et al., 2008; Foxcroft et al.). Piglets with decreased viability from increased litter size are also less prepared to navigate the stress associated with weaning including dietary, environmental, and social changes (Campbell et al., 2013; Moeser et al., 2017). In light of these observations, it is essential to develop nutritional strategies for the sow to allow for support of large litters as well as to allow piglets from larger litters to better navigate stress associated with weaning.

Many feed additives have been considered in sow gestation and lactation diets for the purpose of increasing the health and reproductive efficiency of the sow and recently a promising class of feed additives called postbiotics have emerged. Postbiotics are a preparation of inanimate microorganisms and/or their components that confer a health benefit on the host (Salminen et al., 2021). Postbiotics are feed additives expected to induce changes in the gut microbiome to bring about positive changes on the host. The microbiome of the sow has an impact on productivity; however, the impact of postbiotics on the sow microbiome and litter performance are variable and not well characterized to date (Veum et al., 1995; Kim et al., 2008; Callens et al., 2015; Wang et al., 2018; Costa et al., 2019; Shao et al., 2020; Uryu et al., 2020). It is known that the microbiome plays a

significant role in the health and nutritional status of an animal and that farrowing and lactation place a significant stress on the health and nutritional status of the sow which has the potential to decrease production (Koketsu & Iida, 2017; Shen et al., 2011; Sun et al., 2022; Wang et al., 2019). Investigating how postbiotics influence the sow microbiome is essential to understand how postbiotics may positively impact sow health and production.

While the sow microbiome is important for the health and productivity of the sow, it also plays a large role in piglet health and performance. A sow's health and nutritional status mediates health and development of offspring beginning in utero, throughout lactation, and may continue to have a large impact on post-wean health and performance of subsequent progeny (Vinsky et al., 2006; Yuan et al., 2015). There are two main mechanisms by which the sow impacts piglet health and performance and these are through passive immunity and microbial succession. Passive immunity is the passage of immunoglobulins from maternal milk to the piglet via colostrum and decreased colostrum intake has been shown to have negative effects on piglet survival (Devillers et al., 2011; Quesnel et al., 2012; Rooke & Bland, 2002). Probiotics and prebiotics have been shown to positively impact colostrum quality; however, the impact of postbiotics on colostrum and milk quality has yet to be characterized (Jang et al., 2013; Jurgens et al., 1997; Kogan & Kocher, 2007; Rocha et al., 2022; Zanello et al., 2013). Microbial succession is the seeding of the piglet microbiome via their environment (Nowland et al., 2019a). Piglets are born virtually devoid of microbial species within their gut which is initially populated beginning at birth with milk intake and exposure to their external environment (Gomez-Gallego et al., 2016; Morissette et al., 2018; Nowland et al., 2019b). During and after

parturition, the piglet is exposed to the sow's feces, skin, and mucosal surfaces; therefore, the initial microbiome populations in the piglet are largely dependent upon the composition of the sow's fecal, skin, and mucosal microbiome (Nowland et al., 2019b). In neonatal piglets the gut microbial community is essential for several protective, metabolic, and trophic roles including acting as a barrier against pathogens, aiding digestion and metabolism of colostrum and milk, breaking down toxins and drugs, synthesizing vitamins, absorbing ions, and supporting growth and differentiation of the intestinal epithelium (Nowland et al., 2019b; Yang et al., 2016). Differences in microbiome have been established between high weight gain and low weight gain piglets (Knecht et al., 2020; Morissette et al., 2018). Therefore, there is great potential to have a long-term impact on piglet health and growth performance by influencing the microbiome of the sow.

This study was done due to a lack of research investigating the impact of postbiotic supplementation on the sow microbiome and offspring microbial succession. The objective of this study was to determine the impact of supplementation of a yeast postbiotic in sow gestation and lactation diets on sow reproductive performance, sow microbial communities over time, piglet gut microbial succession into the nursery, and piglet performance.

3.3 Material and Methods

The experimental protocol (#2110-070A) was approved by the South Dakota State University Animal Care and Use Committee and the University of Minnesota Animal Care and Use Committee. The trial followed the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Third Ed., 2010). Two groups were used

to complete the trial, the first ran from January to April, 2022 and the second occurred from March to July, 2022.

3.3.1 Animals and Management

The study was conducted in the farrowing and nursery facilities at the West Central Research and Outreach Center, Morris, MN. A total of 53 multiparous and primiparous females (Topigs Norsvin x Z-line; 239.97 ± 38.82 kg), were used in a randomized incomplete block design from approximately d 80 of gestation up to weaning (20 ± 1 d of lactation). Sows and gilts were relocated from outdoor group housing into the farrowing house at the initiation of supplementation and housed in individual farrowing crates (0.56 m x 2.13 m). Feed was weighed and dispersed by barn staff twice daily, once around 7:30 am and the second allotment around 2:30 pm corresponding with the arrival and departure of barn staff. Females were provided 2.5 kg of feed per day until approximately 15 days before parturition where their daily allotment was increased to 3.6 kg per day. Upon parturition, females were provided *ad libitum* access to feed with fresh feed added twice daily, feeding volume adjusted to reflect the residue of feed remaining in the feeder. Water was provided *ad libitum*. Sows and gilts were supervised during farrowing by the assigned graduate research assistant from the hours of 6am to 6pm in the event farrowing assistance was required. Sows and piglets were checked twice daily by the barn staff and graduate research assistant following the completion of farrowing and up until weaning.

An injection of oxytocin (VetOne, Oxytocin, Boise, ID) was administered to females that had yet to farrow on their expected date at discretion of farm staff. Litters were equalized as close as possible to 12 to 14 pigs within 48 hours by means of cross fostering. Cross

fostering occurred within maternal treatment groups. As soon as piglets were dry or one day post-parturition, animals were processed (individual weights, tail docking, ear-tagging, and castration) and administered a 2 mL intramuscular (i.m.) injection of iron hydrogenated dextran (100 mg/mL, VetOne, Iron Hydrogenated Dextran, Boise, ID). Several young boars who appeared small or thin were processed at 5 to 6 days of age as a measure for reducing stress in the first few days to prevent further health decline. At weaning, all animals were vaccinated with 1 mL i.m. injection of Circumvent PCV-M G2 (Merck Animal Health, Madison, NJ).

At d 20 ± 1 post-farrow, all piglets were weaned and transferred to the nursery. Pigs were allotted to pens within maternal treatment (10 pigs/pen; 12 to 17 pens/maternal treatment; 630 total pigs; 6.18 ± 0.86 kg) and all piglets received the same standard 4-phase nursery diets. All weaned piglets were not placed on trial due to space limitation in the nursery; therefore, piglets were chosen leaving out the least viable animals followed by dividing into 3 weight blocks (Block 1: 3.0 to 5.6 kg; Block 2: 5.6 to 6.6 kg; Block 3: 6.6 to 9.2 kg) and keeping equal numbers of piglets from each weight block for testing. Pens were balanced for weight and litter as much as possible. Weight and feed performance data were recorded during the 42-day nursery test period. Feed and water were offered *ad libitum*. Near termination of the nursery period, piglets were vaccinated orally via water treatment for *Lawsonia Intracellularis* and *Erysepelothrix Rhusiopathiae*. At barn staff discretion, individual piglets were treated for disease or thriftiness with an i.m. injection of Baytril 100 (Elanco, Baytril 100, Greenfield, IN), Dexamethasone (VetOne, Dexamethasone, Boise, ID), or Penicillin (Norocillin; Norbrook, Lenexa, KS) and medication type, dose, and reasoning for treatment were recorded. Pigs who were

removed from the trial due to poor health, death, or euthanized were recorded with date and weight at removal. All pigs and facilities were checked twice daily by trained barn staff and by the assigned graduate research assistant during the course of the study.

3.3.2 Experimental design and dietary treatments

Pregnant females were randomly allotted to one of two experimental diets (n=14-16 animals/treatment/farrowing group), balanced by BW, back caliper, and parity. Dietary treatments were control (CON) and yeast postbiotic (SUP). Control was a standard gestation and lactation diet formulated to meet or exceed nutrient requirements for sows in accordance with NRC (2012; Table 3.1). Yeast postbiotic was added to the CON diet at 0.01% at the expense of corn (Table 3.1). Two sows from control and one from yeast postbiotic were removed from test due to prolonged feed refusal and non-responsiveness to veterinary treatments.

Weaned pigs were provided the same 4-phase nursery pig feeding program where all diets were formulated to meet or exceed NRC (2012) nutrient recommendations for pigs 5 – 20 kg (Table 3.2). Feed budget of each phase was as follows: Phase 1, 0.45 kg/head, Phase 2, 1.81 kg/head, Phase 3, 6.82 kg/head, and Phase 4, roughly 22.7 kg/head or fed until approximately 22.7 kg body weight. Phases 1 and 2 were provided in pellet form with phases 3 and 4 as meal. Water was provided *ad libitum*. Phase 1 consisted of First Feed Pellet - non medicated and Phase 2 consisted of Launch Pellet - non medicated (Vita Plus Corporation, Madison, WI). Phase 3 and 4 diets were standard grind and mix corn/soy diets.

3.3.3 Data collections, chemical analyses, and calculations

Sow BW was recorded at d 85 of gestation (trial start), d 113 of gestation, within 24 hours after parturition, and at weaning. Back fat (BF) at the last rib was measured at d 85 of gestation, d 113 of gestation, and weaning using a back fat caliper. Sow fecal samples were obtained via rectal palpation at d 85 of gestation, d 113 of gestation, within 24 hours after parturition, and at weaning. Fecal samples were collected in labeled 50 mL conical tubes and frozen for further analysis. Litter characteristics (total born, born alive, stillborn, and mummies) were recorded within 24 hours following parturition. Feed orts were weighed at the end of lactation for determination of sow lactation average daily feed intake. Following the completion of the trial, subsequent farrowing characteristics were evaluated.

Piglets were weighed within 24 hours of farrowing, d 7 post-parturition, and at weaning. At d 7, d 14, d 28, and d 42 post-wean, BW of weaned pigs was recorded. In conjunction with weighing, feed disappearance was documented and ADG, ADFI, and G:F was calculated. Three average weight piglets from twelve randomly selected sows per treatment were selected for fecal sampling. Piglet fecal samples were collected via rectal palpation with a damp, sterile cotton swab at d 10 and d 18 post-parturition. Post-wean piglet fecal samples from selected piglets were collected via rectal palpation at d 7, d 14, and d 28 post-wean. All piglet fecal samples were collected in 5 mL microcentrifuge tubes and frozen for further analysis. Feed samples were collected during lactation and the post-wean period.

Following the birth of the first piglet and prior to suckling, colostrum was collected from sows that farrowed between 6 am and 6 pm using gentle stripping from all teats for a total volume of 40 mL in sterile conical tubes (Fisher Scientific, Pittsburgh, PA). Colostrum

was stored at -20ºC until further analysis. Three average weight piglets per litter were selected on d 2 post-parturition for blood collection (1 mL) for assessment of immunocrit and immune status. Blood samples were collected via jugular venipuncture with a 21 ga x 1.5 in needle into a nonheparinized blood collection tube (BD Vacutainer, Franklin Lakes, NJ). Within 24 hours of collection, blood samples were centrifuged at ≥ 5,000 rpm for 10 minutes. Serum was collected and transferred to 5 mL microcentrifuge tubes (Thermo Fisher Scientific, Waltham, MA) and stored at -20ºC for further immune analysis. For analysis, sera samples were thawed and vortexed. Serum and colostrum immunocrit ratio (IR) was determined based on Vallet and Miles (2017) with modification. Sera was subsampled (50 µL) and combined with 40% (wt/vol) ammonium sulfate (1:1 ratio) to precipitate immunoglobulins and vortexed to mix. This solution was loaded into a hematocrit centrifuge tube and centrifuged at 12,000 x g for 10 min at room temperature. Following centrifugation, length of the Ig precipitate and length of the serum solution were measured. Utilizing these measurements, the sera IR was determined by taking precipitate length and dividing by total length of the serum solution. For colostrum analysis, colostrum was thawed and vortexed. Colostrum samples were diluted in a 1:1 ratio with 10% bovine serum albumin (BSA) in 0.9% saline (1 mL BSA: 9 mL saline; Fisher BP6751) and vortexed. In a microcentrifuge tube, 50 µL of diluted colostrum and 50 µL of ammonium sulfate were combined and vortexed to precipitate immunoglobulins. This solution was loaded into hematocrit centrifuge tubes and centrifuged at 12,000 x g for 10 min at room temperature. Following centrifugation, length of Ig precipitate and serum solution were measured, and colostral IR was

determined by dividing precipitate length by serum solution and doubling to account for colostrum dilution.

Sow fecal samples from d 85 of gestation, d 1 of lactation, and weaning were subjected to 16S rRNA sequencing to characterize bacterial species and abundance. The average piglet from each litter that piglets were designated for fecal sampling was selected for 16S rRNA sequencing to characterize bacterial species and abundance. Microbial genomic DNA was isolated from intestinal samples by a repeated bead beating plus column method (Yu and Morrison, 2004), which included the use of the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Fecal material from collections was used as starting material for each microbial genomic DNA preparation. Bead beating was performed twice for each DNA preparation, for a duration of 3 min at 3500 rpm for each repetition. For each sample, approximately 400 ng of amplified DNA were submitted to Molecular Research DNA (MRDNA, Shallowater, TX, USA), which performed all subsequent steps for Next-Generation sequencing, including indexing and library preparation, to generate overlapping paired-end reads with the Illumina MiSeq (2 × 300) platform.

Unless specified, sequence data analysis was performed using custom-written Perl scripts. Raw bacterial 16S rRNA gene V1–V3 amplicon sequences were provided by Molecular Research DNA (MRDNA, Shallowater, TX, USA) as assembled contigs from overlapping MiSeq (2 × 300) paired-end reads from the same flow cell clusters. Reads were then selected to meet the following criteria: the presence of both intact 27F (forward) and 519R (reverse) primer nucleotide sequences, a length between 400 and 580 nt, and a minimum quality threshold of no more than 1% of nucleotides with a Phred quality score lower than 15 (Opdahl et al., 2018; Poudel et al., 2020).

Following quality screens, sequence reads were aligned, then clustered into Operational Taxonomic Units (OTUs) at a genetic distance cutoff of 5% sequence dissimilarity (Opdahl et al., 2018; Poudel et al., 2020). The OTUs were screened for DNA sequence artifacts using the following methods. Chimeric sequences were first identified with the 'chimera.uchime' (Edgar et al., 2011) and 'chimera.slayer' (Haas et al., 2011) commands from the MOTHUR (version 1.44.1) open-source software package (Schloss et al., 2009). Secondly, the integrity of the 50 and 30 ends of OTUs was evaluated using a database alignment search-based approach; when compared to their closest match of equal or longer sequence length from the NCBI 'nt' database, as determined by BLAST (Altschul, 1997), OTUs with more than five nucleotides missing from the 50 or 30 end of their respective alignments were discarded as artifacts. Single read OTUs were subjected to an additional screening, where only sequences that had a perfect or near-perfect match to a sequence in the NCBI 'nt' database were kept for analysis, i.e., that the alignment had to span the entire sequence of the OTU, and a maximum of 1% of dissimilar nucleotides was tolerated.

After removal of sequence chimeras and artifacts, OTUs were subjected to taxonomic assignments as follows: two general taxonomic level assignments (Phylum and Family) for all OTUs using RDP Classifier (Wang et al., 2007), and closest relative identification for select OTUs using BLAST queries (Altschul, 1997). Alpha diversity indices (Observed OTUs, Chao, Ace, Shannon, and Simpson) were determined using the 'summary.single' command from MOTHUR (version 1.44.1) (Schloss et al., 2009) on a dataset subsampled to 5000 reads for each sample. Principle Coordinate Analysis (PCoA) for beta diversity was performed using the same rarefied dataset, by determining Bray–

Curtis distances with the ‘summary.shared’ command followed by the ‘pcoa’ command in MOTHUR (version 1.44.1) (Schloss et al., 2009).

3.3.4 Statistical Analysis

Data was analyzed using the mixed model procedure of SAS (Version 9.4, SAS Inst. Inc., Cary, NC) considering the effect of dietary supplementation where the sow was the experimental unit and sow (block) and parity as random effects during the farrowing and suckling period. In the post-wean period, performance was analyzed as a repeated measures nested design with pen nested in sow treatment as the random variable. Variables of particular interest included sow reproductive performance (i.e. litter size, lactation feed intake) and piglet performance (i.e. nursery feed intake, growth rate during lactation and in the nursery). Significant differences were reported at $P < 0.05$ and tendencies for significance were reported when $0.05 \leq P \leq 0.10$.

Comparisons of abundance for bacterial taxonomic groups and OTUs amongst different dietary treatments were performed in R (Version R-3.6.2) using the non-parametric test Kruskal–Wallis (command ‘kruskal.test’), followed by the Wilcoxon test (command ‘pairwise.wilcox.test’) for multiple pairwise comparisons, which included the Benjamini-Hochberg correction to control for false discovery rate. For alpha diversity indices, normal distribution of data was first confirmed using the Shapiro Wilk test (command ‘shapiro.test’), then comparison across the different diet groups was performed using ANOVA followed by Tukey’s range test for multiple comparisons; these tests were conducted using R (Version R-3.6.2). Statistical significance was set at $P \leq 0.05$.

PERMANOVA (permutational multivariate analysis) was performed in R (Version R3.6.2) using the command ‘adonis’, followed by the command ‘pairwise.adonis’ to identify pairs of sample groups that were different. For all analyses, tests resulting in $P \leq 0.05$ were considered significant. Analysis by LDA Effect Size (LEfSe) [56] was performed using a publicly available online implementation of the program (https://huttenhower. sph.harvard.edu/galaxy/ accessed on 16 October 2020).

3.4 Results

In general pigs were healthy and there were few veterinary treatments during the entire experimental period as a result no statistical assessment of veterinary treatment was conducted.

3.4.1 Sow Performance

A parity x treatment interaction, where control sows gained less weight in the last 35 days of gestation than supplemented sows, was observed for sow body weight gain and loss during gestation ($P < 0.05$; Table 3.3). A similar effect in which supplemented sows had higher feed intake than control sows was noted in sow lactation average daily feed intake. There was no effect of the yeast postbiotic, parity, or their interaction on sow reproductive performance (Table 3.3).

3.4.2 Piglet Performance

There was no significant effect of treatment noted on suckling piglet growth performance or on piglet immunocrit (Table 3.3). There was no effect of treatment on any piglet performance parameters throughout the six-week nursery period (Table 3.4). There was

an effect of period where piglets increased ($P < 0.001$) in weight, ADG, and feed efficiency over time. There was no interaction between treatment and period.

3.4.3 Taxonomic Composition Analysis of Fecal Bacterial Communities

A total of 3,818,893 quality filtered sequence reads were used for the composition analysis described in this report (22,868 reads per sample). Across all sow fecal samples, five predominant phyla (*Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Spirochaetes*, and unclassified Phyla) were identified. *Firmicutes* was the most abundant phylum, showing increasing relative abundance from d85 of gestation to wean (Figure 3.1, Table 3.5; $P < 0.05$). The most abundant *Firmicutes* families, *Clostridiaceae* 1 and *Peptostreptococcaceae*, were not different between treatment groups, with both families remaining the dominant family throughout the experimental period (Table 3.5, Figure 3.1). Likewise, across all piglet fecal samples, five prevalent phyla (*Firmicutes*, unclassified phyla, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria*) were isolated. Similar to sow samples, phylum *Firmicutes* was most abundant in piglet fecal microbial populations and showed increasing relative abundance from D18 of lactation to post-wean day 28 (PWD28; Figure 3.2, Table 3.6; $P < 0.05$). While there were no differences between treatment groups, several interesting shifts in piglet fecal microbial populations were noted over time. A phylum of unclassified bacteria comprised a large relative abundance on D18 of lactation constituting a similar percentage of bacterial populations as *Firmicutes* (Figure 3.2, Table 3.6). Two *Proteobacteria* families, *Yersiniaceae* and *Moraxellaceae*, and one *Actinobacteria* family, *Propionibacteriaceae*, had relatively greater relative abundance at PWD7 compared to all other time points (Figure 3.2, Table 3.6).

3.4.4. Alpha Diversity

Because taxonomic profiling indicated differences in composition associated with time, OTU-level analyses were performed to gain further insight (Table 3.9 and 3.10). Based on the alpha diversity indices Observed OTUs, Ace, Chao, and Shannon, showed consistent bacterial diversity across time in sows ($P > 0.05$). These values appeared to increase with time in piglets except for Simpson's index which decreased ($P \leq 0.05$; Table 3.7 and 3.8). Clustering samples by time points showed clear shifts in microbial communities over time for both the sows and piglets (Figure 3.3 and 3.4) and was supported by the statistical PERMANOVA test ($P = 0.001$).

3.4.5. OTU Composition Analysis

Of the 14,924 OTUs that were identified across all samples, the most abundant OTUs, defined as representing at least 3.0% of sequences in at least one set of samples, were further analyzed (Table 3.9; Table 3.10). For instance, >JK_30-00008, >JK_16-00021, and >JK_23-00527 were the most highly represented sow OTUs of the phylum *Firmicutes*, representing a high proportion of sequence reads from this taxonomic group across all samples and most OTUs either remained consistent or decreased in abundance over time. Piglet OTU compositions were dominated by *Firmicutes* and unknown bacteria. Interestingly a spike in a couple OTUs (>JK_137-00038 and >JK_134-00239) belonging to *Proteobacteria* was noted at PWD 7 while being virtually absent at all other time points.

3.5 Discussion and conclusion

The objective of this study was to assess the impact of yeast postbiotic supplementation in late gestation and lactation diets on sow reproductive performance, suckling piglet performance, piglet performance post-wean through the nursery period, sow fecal bacterial compositions, and piglet fecal bacterial compositions through the nursery. There was limited impact of the yeast postbiotic on performance results. Sow reproductive performance was not impacted by supplementation with the postbiotic, while an increase in sow lactation feed intake was noted in sows supplemented with the postbiotic. Feed intake during gestation and lactation is critical in order for a sow to produce and support a large litter (Kim et al., 2013; Costa et al., 2019) Lactation feed intake may be a limiting factor in some cases for sows lactating large litters, therefore increasing dietary nutrient concentrations or strategies to increase lactation feed intake are imperative (Kim et al., 2013). An impact on sow lactation feed intake was not observed in a similar study by Shen et al. (2011). This same study reported an increase in litter weight and litter weight gain during lactation; and both studies reported no increase in reproductive performance when supplementing a postbiotic (Veum et al., 1995; Shen et al., 2011).

With respect to offspring, piglet suckling performance and immunocrit was not improved with postbiotic supplementation, similarly with piglet performance in the post-weaning period. A similar study reported no effect on piglet growth performance through the nursery and finisher period when supplementing a postbiotic to either the sow or the piglet (Shen et al., 2017). The current study is unique from many other studies in that supplementation was limited to the sow with no supplementation in piglet diets while many studies provided supplemented diets to both sows and piglets post-wean. Several

studies reported increased performance of piglets, especially in the nursery phase, when diets were supplemented with a yeast postbiotic; therefore, the lack of performance differences in the current study may be due to diet supplementation being too far removed the piglets time in the nursery (Shen et al., 2009; van der Peet-Schwering et al., 2007). Differences in supplementation lengths may have played a role in the lack of differences during the suckling period and into the post-wean piglets' performance with the current study supplementing during the last portion of gestation and throughout lactation in comparison to longer supplementation periods in other similar trials (Shen et al., 2011; Veum et al., 1995). A lack of difference in performance in this study may also be attributed to utilization of different supplementation levels in the current trial compared others (Shen et al., 2011; Veum et al., 1995). It may be that a postbiotic supplementation level of 0.5% in the sow diet may be too low in order to elicit an effect, particularly in the offspring. Studies investigating postbiotics in sow diets have utilized a somewhat similar level with no effects; however, studies in post-wean piglets have observed significant performance benefits with an increased supplementation level (Shen et al., 2009, 2011; van der Peet-Schwering et al., 2007; Veum et al., 1995). In addition, studies utilizing probiotics typically supplement at 0.02% - 0.5% of the diet while observing significant impacts on performance and reproduction which means the lack of differences in the current study may reflect a necessary increase in supplementation level for postbiotics to garner similar effects to probiotics (Elghandour et al., 2020; Hayakawa et al., 2016). These considerations may similarly explain the lack of difference in piglet immunocrit values.

There were no significant effects of treatment on fecal bacterial composition of sows. This lack of change may be due to dose response level or treatment period as noted above as well as due to the myriad of factors which play into shifting specific gut bacterial communities. Due to the large number of factors which influence the gut microbiota, including age, genetics, environment, and nutrition, studying specific changes in the gut microbiome as a result of a specific dietary treatment or physiological status (i.e. stage of pregnancy) can be difficult (Gaukroger et al., 2021; Liu et al., 2019). In spite of the plethora of factors mentioned above which play into gut microbial communities, time remains one of the largest drivers of change in the gut microbiome (Gaukroger et al., 2021; Kim et al., 2011; Liu et al., 2019). Similar to previous reports, the current trial observed shifts of the gut microbiota over time in both sows and piglets. Consistent with other studies, the fecal bacterial composition of sows were dominated by species of *Firmicutes* and *Bacteroidetes* phyla (Kim et al., 2011; Mach et al., 2015; Qiu et al., 2018). However the current study observed an increase of *Firmicutes* in sow feces from late gestation to the end of lactation while other studies have reported a relatively consistent population of these microorganisms over the same time period (Gaukroger et al., 2021; Liu et al., 2019).The discrepancy in *Firmicutes* population may possibly be due to fewer time points to capture all changes in the current study. Interestingly, the current trial observed a much higher relative abundance of *Firmicutes* than *Bacteroidetes* in the fecal microbiome which was also shown by Liu et al. (2019); however, a relatively equal relative abundance of *Bacteroidetes* and *Firmicutes* has also been reported (Gaukroger et al., 2021). This difference in relative levels of *Firmicutes* versus *Bacteroidetes* could be due to dietary or environmental differences between trials. A slight decrease in relative

abundance of *Spirochaetes* from CON d85 at 6.09% and SUP d85 at 7.53% to CON Wean at 3.05% and SUP Wean at 3.97% was observed in the current study and is supported by other research (Liu et al., 2019; Gaukroger et al., 2021) Although not observed in this study, the relative abundance of *Spirochaetes* has been noted to increase just after farrowing (Liu et al., 2019; Gaukroger et al., 2021). Consistent with other studies, a definitive change in the microbiome from gestation to the periparturient period was noted in this study (notably a decrease in known commensal family *Lactobacillaceae*) which can likely be attributed to metabolic syndrome in sows from increasing demands for fetal growth as well as stress associated with pregnancy (Liu et al., 2019; Gaukroger et al., 2021). The changes in sow microbiome over pregnancy and lactation is not well understood and requires more study to elucidate probable agents of change. Once causes of change to the sow microbiome over gestation and lactation are understood then it may be possible to manipulate the gut microbiota to partially alleviate stress of pregnancy and lactation on the sow to increase sow longevity and productivity. For example, the family *Spirochaetaceae,* which is a bacterial family associated with several diseases including swine dysentery, was observed to decrease throughout gestation and lactation in this study. Investigating how to lower the relative abundance of this family to a greater extent in sows could be a possible opportunity to improve their health status (Karami et al., 2014). The family *Ruminococcaceae* increased throughout gestation and lactation which may be desirable as members of this family have been identified as short chain fatty acid producers which play into intestinal health (Xie et al., 2022). Lastly, the family *Clostridiaceae 1* increased in relative abundance with time in sow feces as well. Although the effects of this increase in *Clostridiaceae* 1 are unknown,

this family is known to contain several pathogenic species and has been associated with increased cecum succinate concentrations in rats (Tulstrup et al., 2015). Microbiota-derived gut succinate has been associated with both positive and negative effects in human health; thus, this underscores the need to understand shifts in the sow microbiome throughout this gestation and lactation to better understand their impact on her health and productivity (Fernández-Veledo and Vendrell, 2019).

Similar to sow fecal bacteria analysis, there were no significant differences observed between treatments in piglet fecal bacterial composition. This lack of difference is likely due to similar reasons discussed above related to dose response level or treatment period. A trend of increasing alpha diversity indices observed in the current study is consistent with other research that report alpha community diversity and richness increase primarily in the first 21 days post-wean and possibly until market (Frese et al., 2015; Kim et al., 2011; Lu et al., 2018; Wang et al., 2019). In the current study there were several intriguing shifts in microbial communities in piglets after weaning, in particular, bacterial populations at D18 of lactation and PWD 7. Fecal bacteria were dominated largely by *Firmicutes* and *Bacteroidetes* on PWD14 and PWD28 and is similar to dominant bacterial phyla reported in prior research (Lu et al., 2018; Wang et al., 2019). However, at D18 of lactation the highest proportion of bacteria fell into an unclassified bacteria category which was not observed in other studies investigating the changes in the piglet microbiome around weaning (Kim et al., 2011; Lu et al., 2018; Wang et al., 2019). Another intriguing shift observed at PWD7 was a sharp decline in the prevalence of unclassified bacteria and *Bacteroidetes* to a piglet bacterial composition dominated by *Proteobacteria* and *Actinobacteria*. This shift was also not reported in other studies (Kim

et al., 2011; Frese et al., 2015; Lu et al., 2018; Wang et al., 2019). A decline in *Bacteroidaceae* and an increase in *Prevoteallaceae* has been reported as piglets change from a milk-based diet to a plant-based diet (Frese et al., 2015; Wang et al., 2019). The significance of the specific shifts at PWD7 is very important given this was the first fecal sampling post-wean and therefore reflects the influence of weaning stress on the piglet's gut bacteria. The challenge with associating a physiological outcome of these shifts in the microbiome is the role of specific groups of microorganisms in health, metabolism, and disease is unclear and many times depends on the context in which the piglet is living. The second challenge in inferring the role of microorganisms is the lack of cultured microorganisms which is reflected by the number of uncultured microorganisms in the OTU taxonomic analysis of this study. However, knowledge of gut microorganisms is advancing and therefore this discussion will present the general understanding of what the abnormal shifts in the piglet microbiome could mean. The first major shift from D18 to PWD7 includes the family *Lachnospiraceae* which has both beneficial and detrimental roles in the gut including producing short chain fatty acids, being associated with anti-inflammatory properties, and being increased during incidences of inflammatory bowl disease (IBD) and other metabolic disorders (Vacca et al., 2020). The second major shift during this time period was in the family *Erysipelotrichaceae.* This family also possesses species which can have both beneficial and detrimental effects depending on the physiological context of the gut. Species of this family appear to be involved in host lipid metabolism and some species are increased during gut inflammation and other GIT disorders while others appear to provide immunological benefits (Kaakoush, 2015). The third major shift in fecal microbial abundance from D18 of lactation to PWD7 bacterial

compositions was a sharp increase in the *Proteobacteria* family, *Yersiniaceae.* This is a family in which most members are not well characterized; however, the species which have been investigated are understood to be pathogenic organisms causing multiple zoonotic diseases including plague and enteritis (Barbierifon et al., 2020; Dheyab, 2022; Naktin & Beavis, 1999). An increase in another family of *Proteobacteria*, *Moraxellaceae*, was also observed during this time period in the piglet's fecal bacteria composition although to a lesser magnitude than *Yersiniaceae*. *Moraxellaceae* has been observed in increased concentrations in airways of asthmatic individuals and the most understood well studied genus (*Moraxella)* of this family is well characterized as a human respiratory tract pathogen (Kennedy et al., 2020; Liu et al., 2020; Liu et al., n.d.). The last major shift in piglet fecal bacteria from D18 to PWD7 is an increase in the family *Propionibacteriaceae*. This family is likewise not well characterized but probably contains mostly detrimental microorganisms (Dworkin et al., 2006; Schaal et al., 1980). As previously mentioned the significance of the shifts in bacteria during this time are not always straightforward; however, the majority of these shifts appear to be towards species of microorganisms associated with an inflammatory state or negative to health which reflects the effects of weaning stress on these piglets. This underscores the need to understand the significance of these species to devise solutions for mitigating these shifts so piglets can better navigate weaning associated stress.

In conclusion, there was limited impact of the yeast postbiotic supplementation in maternal diets which may be due to several reasons; however, shifts in gut microbial populations in sows and piglets over time were observed. These shifts, particularly in the sow fecal bacteria populations after parturition and piglet fecal bacteria composition

around weaning were intriguing. The lack of definitive conclusions due to a lack of understanding of the significance in specific microbial populations highlights the need for more investigation into this area. Unraveling the importance and function of the specific groups of microorganisms outlined in this discussion may provide the key to navigating weaning stress or minimizing stress on the sow following parturition.

Table 3.1. Control (CON) and Supplemented (SUP) sow gestation and lactation diet formulations

Item, kg	Gestation		Lactation	
	CON	SUP	CON	SUP
Corn	760.7	742.5	619.1	600.9
Soybean meal 46%	86.4	86.4	209.1	209.1
TNI Super Sow H.A. Premix	39.1	39.1	39.1	39.1
Distillers Corn oil	-	-	18.2	18.2
Preblend	23.0*	23.0*	23.6**	23.6**
SDSU Postbiotic Premix	-	18.2	-	18.2
Total	909.2	909.2	909.1	909.1
Calculated analysis:				
ME, kcal/kg	3249.4	3249.4	3334.1	3334.1
Crude protein, %	11.4	11.4	16.7	16.7
Lysine, total %	0.68	0.68	1.09	1.09
Calcium, %	0.86	0.86	0.91	0.91
Phosphorus, %	0.65	0.65	0.70	0.70
Salt, %	0.51	0.51	0.51	0.51

*Preblend contains 22.7 kg soybean meal + 0.23 kg L-Lys HCl.

**Preblend contains 22.7 kg soybean meal + 0.91 kg L-Lys HCl

Table 3.2. Phase 3 and Phase 4 nursery diet formulations

Item, kg	Nursery Diet	
	Phase 3	Phase 4
Corn	490.9	588.6
Soybean meal 46%	227.3	277.3
TNI 400 Nursery Base	181.8	-
TNI 25-80 NG Premix	-	34.1
Soy Oil	9.1	9.1
Total	909.1	909.1
Calculated Analysis:		
Dry Matter, %	89.1	88.1
ME, kcal/kg	3345.8	3338.9
Crude protein, %	21.03	19.51
Fat %	4.41	4.42
Fiber %	2.00	2.42
Lysine, total %	1.54	1.38
SID Lysine %	1.39	1.25
Calcium, %	0.70	0.62
Phosphorus, %	0.64	0.55
Ca:P Ratio	1.10	1.13
Salt, %	0.65	0.59

Table 3.3. Main effects of yeast postbiotics on sow reproductive performance.

	Dietary treatments		SEM	*P*-value			
Item	CON	SUP		Group	Parity[1]	Trt	ParityxTrt
# of sows	26	27					
Sow BW,kg							
BW d80	243.2	238.9	7.331	0.669	0.024	0.680	0.209
BW d113	266.8	263.0	6.166	0.193	0.042	0.666	0.377
BW d2	245.5	243.2	5.756	0.105	0.030	0.782	0.257
BW Wean	240.8	236.6	7.140	0.108	0.010	0.685	0.104
Sow BW Dif, kg							
Dif d113-80	23.5	24.1	1.661	0.004	0.014	0.827	0.025
Dif wean-d2	-5.3	-6.6	2.831	0.511	0.023	0.751	0.091
Sow Caliper							
d80	15.9	15.5	0.253	0.415	0.150	0.343	0.921
d113	15.0	15.1	0.256	0.0002	0.0002	0.939	0.843
Wean	14.5	14.1	0.337	0.023	0.892	0.429	0.185
Sow LacADFI, kg	6.4	6.8	0.209	0.313	0.0002	0.225	0.043
Reproduction							
Born alive	14.4	14.1	0.540	0.656	0.099	0.764	0.839
Stillborn	1.2	1.3	0.260	0.896	0.909	0.828	0.807
Total born	16.1	15.7	0.627	0.911	0.131	0.634	0.763
Mummies	0.2	0.2	0.098	0.107	0.996	0.841	0.793
Pigd2[1]	14.1	14.0	0.533	0.710	0.113	0.888	0.962
Dead[2]	0.2	0.1	0.102	0.678	0.629	0.393	0.405
MORtod2[3]	1.8	0.8	0.786	0.572	0.550	0.380	0.320
PigsACF[4]	14.0	14.0	0.338	0.769	0.278	0.963	0.982
MORovr[5]	7.7	8.9	1.922	0.684	0.481	0.663	0.319
WeanedCF[6]	13.0	12.9	0.299	0.521	0.509	0.830	0.509
WeanedOrg[7]	13.0	12.9	0.466	0.688	0.095	0.897	0.772
Suckling, kg							
BW Birth[8]	1.45	1.48	0.047	0.838	0.786	0.692	0.970
BW d7	2.67	2.82	0.080	0.538	0.877	0.201	0.906
BW Wean	6.04	6.29	0.167	0.665	0.894	0.292	0.586
ADG	0.22	0.24	0.010	0.867	0.963	0.583	0.834
Immunocrit							
Serum ratio[9]	0.168	0.174	0.005	0.681		0.413	
Colostrum ratio[10]	0.265	0.284	0.030			0.691	

[1]Number of pigs per sow on d2 lactation
[2]Piglets dead in the first 2 days of lactation
[3]Piglet mortality (%) in the first 2 days of lactation
[4]Number of pigs per sow after cross-fostering
[5]Piglet mortality (%) from day 2 of lactation until weaning (overall mortality)
[6]Number of piglets weaned per sow from litters considering cross-fostering
[7]Number of piglets weaned per sow from litters not considering cross-fostering
[8]Born alive birth weight
[9]Data not blocked by parity due to number of observations(n=53)
[10]Data not analyzed considering group or block due to low # of observations (n=12 total)

Table 3.4. Main effects of yeast postbiotics on piglet post-wean performance.

	Dietary treatments			*P*-value			
	CON	SUP	SEM	Block[1]	Period[2]	Trt	Trt*Period
# of pens[3]	32	31					
Body Weight, kg			0.244	0.950	<.0001	0.730	0.960
BW d0	6.18	6.16					
BW d7	6.82	6.91					
BW d14	8.21	8.31					
BW d28	13.89	13.76					
BW d42	21.56	21.80					
ADG, kg			0.010	0.833	<.0001	0.500	0.228
ADG d7	0.088	0.096					
ADG d14	0.211	0.205					
ADG d28	0.397	0.387					
ADG d42	0.540	0.569					
ADFI, kg			0.012	0.995	<.0001	0.886	0.610
ADFI d7	0.128	0.137					
ADFI d14	0.282	0.269					
ADFI d28	0.547	0.540					
ADFI d42	0.844	0.859					
G:F, kg			0.026	0.942	<.0001	0.571	0.827
G:F d7	0.658	0.688					
G:F d14	0.769	0.769					
G:F d28	0.726	0.713					
G:F d42	0.639	0.665					
F:G, kg			0.063	0.348	0.0012	0.627	0.916
F:G d7	1.618	1.556					
F:G d14	1.374	1.357					
F:G d28	1.384	1.411					
F:G d42	1.553	1.516					

[1]Piglets blocked by group
[2]Weigh periods
[3]Nursery pen with 10 pigs/pen

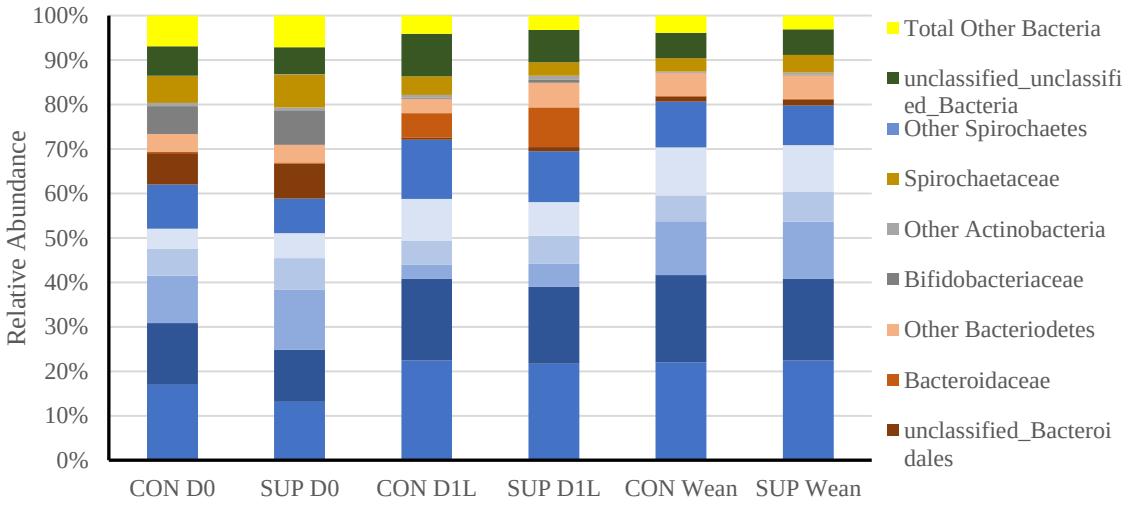


Figure 3.1. Taxonomic profile at the phylum and family level of fecal bacterial communities of sows provided gestation and lactation diets supplemented (SUP) or non-supplemented (CON) with yeast postbiotic. Families belonging to the same phylum are represented by different shades of the same color: Firmicutes (blue), Bacteroidetes (red), and unclassified bacteria (green).

Table 3.5. Mean relative abundance (%) of main bacterial groups in sow control (CON) and treatment (SUP)[1].

	Day 90 of gestation		Day 1 of lactation		Weaning	
Taxon	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**
Firmicutes	62.03	58.82	72.06	69.47	80.64	79.80
Clostridiaceae 1	16.94	13.14	22.40	21.67	21.97	22.39
Peptostreptococcaceae	13.89	11.70	18.46	17.33	19.66	18.45
Lactobacillaceae	10.66	13.53	3.07	5.24	12.11	12.81
Lachnospiraceae	6.05	7.07	5.46	6.24	5.82	6.77
Ruminococcaceae	4.55	5.59	9.35	7.55	10.80	10.43
Other Firmicutes	9.94	7.79	13.32	11.45	10.27	8.94
Bacteriodetes	11.35	12.08	9.23	15.45	6.43	6.75
unclassified_Bacteroidales	7.00	7.87	0.47	0.95	1.15	1.29
Bacteroidaceae	0.39	0.26	5.53	8.90	0.20	0.22
Other Bacteriodetes	3.96	3.95	3.23	5.61	5.08	5.24
Actinobacteria	6.98	8.41	0.88	1.51	0.34	0.66
Bifidobacteriaceae	6.23	7.77	0.21	0.61	0.05	0.11
Other Actinobacteria	0.76	0.64	0.67	0.91	0.29	0.55
Spirochaetes	6.09	7.53	4.19	3.02	3.05	3.97
Spirochaetaceae	6.06	7.45	4.19	3.02	3.05	3.97
Other Spirochaetes	0.03	0.08	0.00	0.00	0.01	0.00
unclassified_unclassified_Bacteria	6.65	6.05	9.52	7.33	5.66	5.71
Total Other Bacteria	6.89	7.11	4.12	3.20	3.88	3.10

[1]Yeast postbiotic supplemented at 0.5% and 0.2% in sow gestation and lactation diets respectively. Supplementation from d85 of gestation until weaning

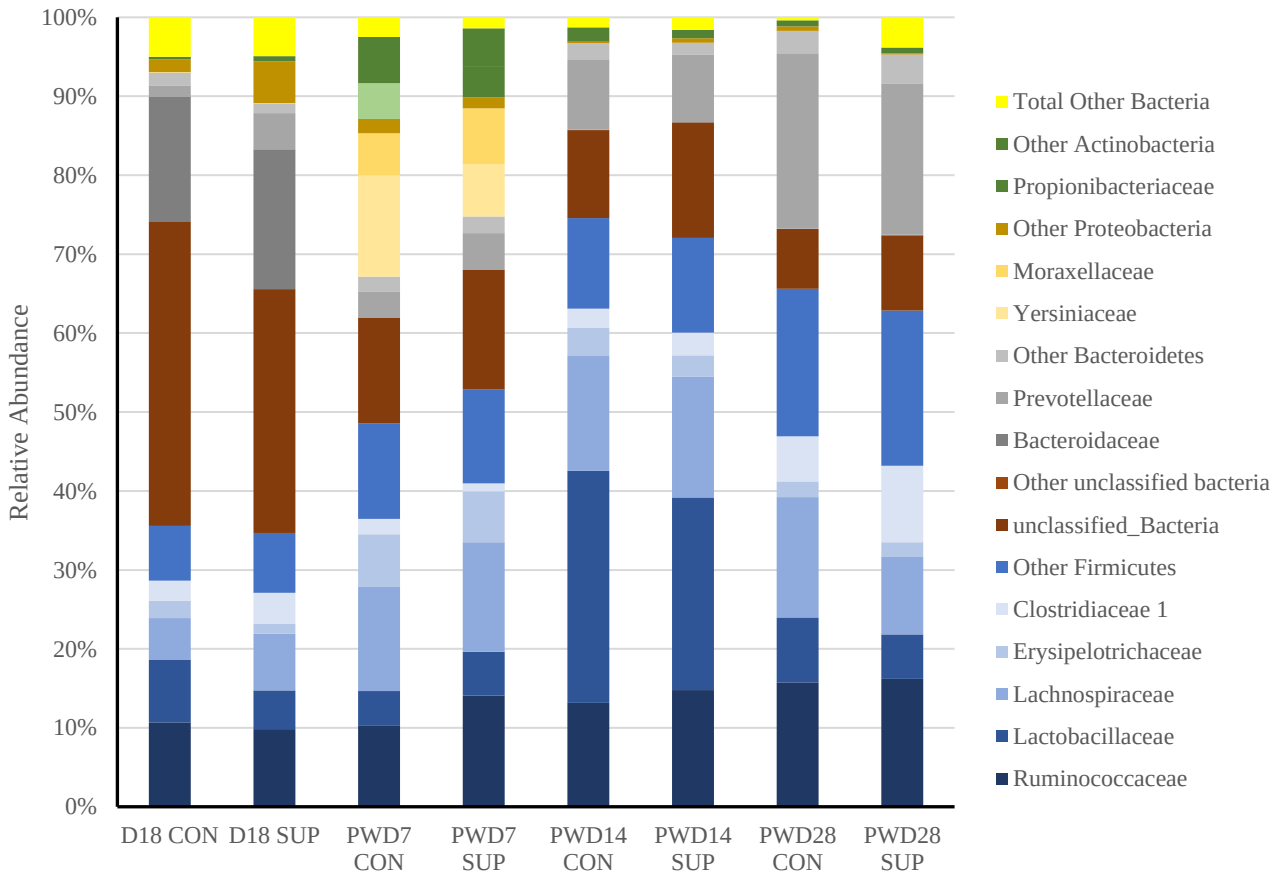


Figure 3.2. Taxonomic profile at the phylum and family level of fecal bacterial communities of piglets from sows provided gestation and lactation diets supplemented (SUP) or non-supplemented (CON) with yeast postbiotic. Families belonging to the same phylum are represented by different shades of the same color: Firmicutes (blue), unclassified bacteria (red), and Bacteroidetes (grey).

Table 3.6. Mean relative abundance (%) of main bacterial groups in piglet control (CON) and treatment (SUP).[1]

	D18		PWD7		PWD14		PWD28	
Taxon	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**
Firmicutes	35.60	34.70	48.60	52.86	74.55	72.07	65.63	62.84
Ruminococcaceae	10.69	9.83	10.31	14.08	13.17	14.78	15.76	16.19
Lactobacillaceae	7.89	4.90	4.34	5.56	29.39	24.42	8.23	5.66
Lachnospiraceae	5.34	7.18	13.25	13.88	14.63	15.25	15.24	9.80
Erysipelotrichaceae	2.15	1.30	6.62	6.43	3.52	2.73	1.99	1.86
Clostridiaceae 1	2.58	3.91	1.96	1.00	2.40	2.88	5.69	9.68
Other Firmicutes	6.94	7.57	12.11	11.91	11.44	12.02	18.72	19.64
unclassified_unclassified_Bacteria	38.56	30.87	13.32	15.15	11.17	14.61	7.55	9.54
unclassified_Bacteria	38.56	30.87	13.32	15.15	11.17	14.61	7.55	9.54
Other unclassified bacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacteriodetes	18.82	23.49	5.24	6.75	11.01	10.09	25.11	22.90
Bacteroidaceae	15.83	17.73	0.10	0.07	0.05	0.03	0.07	0.10
Prevotellaceae	1.34	4.58	3.26	4.57	8.83	8.53	22.20	19.16
Other Bacteroidetes	1.65	1.19	1.88	2.11	2.13	1.53	2.85	3.63
Proteobacteria	1.72	5.32	20.08	15.09	0.20	0.56	0.57	0.17
Yersiniaceae	0.05	0.05	12.85	6.67	0.01	0.00	0.00	0.00
Moraxellaceae	0.02	0.03	5.32	7.02	0.01	0.00	0.00	0.00
Other Proteobacteria	1.65	5.24	1.91	1.40	0.18	0.56	0.57	0.17
Actinobacteria	0.30	0.68	10.28	8.77	1.78	1.07	0.77	0.74
Propionibacteriaceae	0.03	0.05	4.45	3.98	0.00	0.00	0.00	0.00
Other Actinobacteria	0.28	0.64	5.83	4.79	1.78	1.07	0.77	0.74
Total Other Bacteria	5.01	4.94	2.49	1.38	1.28	1.59	0.37	3.82

[1]Yeast postbiotic supplemented at 0.5% and 0.2% in sow gestation and lactation diets respectively. Supplementation from d85 of gestation until weaning

Table 3.7. Observed OTUs and alpha-diversity indices in 2 sow dietary treatment groups across time. Values are shown as means.

Item	CON D0	SUP D0	CON D1L	SUP D1L	CON Wean	SUP Wean
OTUs	370.9	375.8	347.5	361.8	386.6	377.3
Ace	1879.3	1963.7	1722.5	1798.4	2042.6	1770.9
Chao	1066.2	1062.1	992.3	995.2	1087.7	1031.2
Shannon	3.95	3.93	3.92	3.99	4.05	4.01
Simpson	0.07	0.08	0.07	0.07	0.07	0.07

Table 3.8. Observed OTUs and alpha-diversity indices in 2 piglet dietary treatment groups across time. Values are shown as means.

Item	D18 CON	D18 SUP	PWD7 CON	PWD7 SUP	PWD14 CON	PWD14 SUP	PWD28 CON	PWD28 SUP	P-value
OTUs	181.7^{d}	177^{d}	283.8^{cd}	315.2^{cd}	365.5^{bc}	354.4^{c}	497.7^{ab}	517.8^{a}	<0.001
Ace	836.6^{d}	652.3^{d}	1269.5^{cd}	1370.2^{bcd}	1728.1^{abcd}	1815.5^{abc}	2218.2^{a}	2079.3^{ab}	<0.001
Chao	462.7^{cd}	418.1^{d}	733.5^{cd}	803.7^{bc}	1005.1^{ab}	972.8^{ab}	1285.2^{a}	1253.3^{a}	<0.001
Shannon	2.72^{c}	2.75^{c}	3.64^{c}	3.91^{bc}	3.96^{bc}	3.96^{bc}	4.79^{ab}	4.98^{a}	<0.001
Simpson	0.21^{a}	0.22^{a}	0.10^{b}	0.07^{b}	0.10^{b}	0.10^{b}	0.04^{b}	0.03^{b}	<0.001

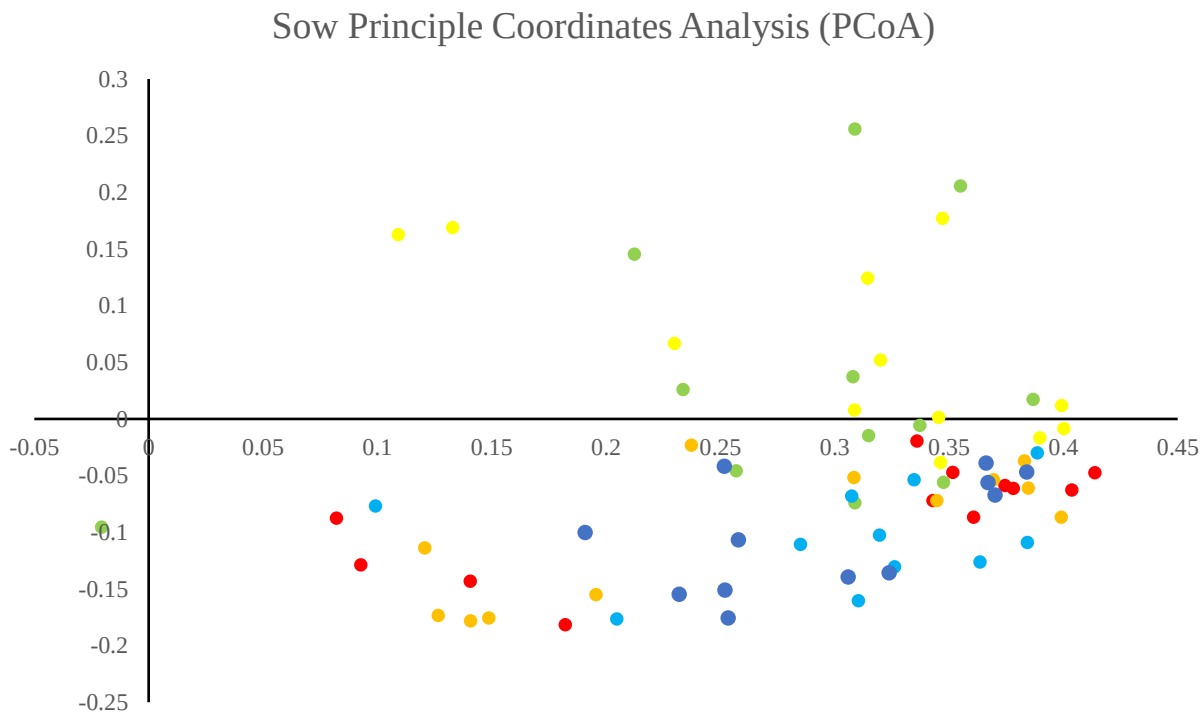


Figure 3.3. Time x Treatment Sow PCoA. Sow D0 CON (red), D0 SUP (orange), D1L CON (yellow), D1L SUP (green), Wean CON (light blue), and Wean SUP (dark blue)

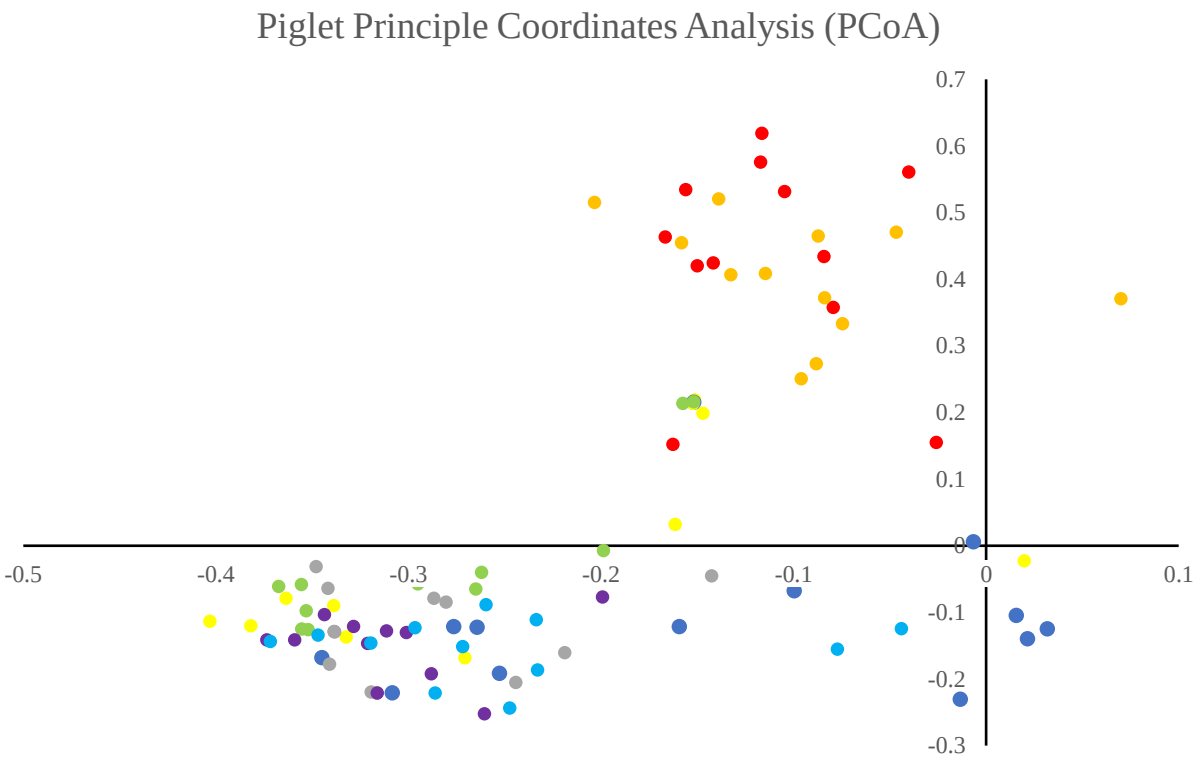


Figure 3.4. Time x Treatment Piglet PCoA. Piglet D18 CON (red), D18 SUP (orange), PWD7 CON (yellow), PWD7 SUP (green), PWD14 CON (grey), PWD14 SUP (purple), PWD 28 CON (light blue), and PWD 28 SUP (dark blue)

Table 3.9. Mean relative abundance of the main bacterial OTUs identified in sows. Abundance is presented as a percentage (%) of the total number of analyzed reads per sample.

	D0		D1L		Wean		
OTU	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**	**Closest Taxon (id%)**
Firmicutes							
>JK_30-00008	9.01	12.04	1.00	3.03	9.41	9.74	Lactobacillus amylovorus (99.8)
>JK_16-00021	10.60	8.98	15.05	13.65	15.61	14.47	Uncultured Peptostreptococcaceae (99.6)
>JK_23-00527	6.65	5.37	8.88	7.03	8.23	7.79	Clostridium sp. DSM 107452 (99)
>JK_14-00443	6.26	5.09	2.86	3.86	4.35	3.99	Uncultured Clostridium sp. (99.6)
>JK_33-00585	0.87	0.58	1.05	1.85	4.61	6.36	Uncultured Clostridum (99.4)
Bacteroidetes							
>JK_38-00035	0.13	0.04	4.62	8.74	0.15	0.16	Bacteroides fragilis (99.6)
>JK_10-00026	2.59	3.48	0.05	0.10	0.07	0.10	Uncultured Bacteroidales (98.9)
Actinobacteria							
>JK_8-00041	5.44	6.88	0.03	0.24	0.03	0.04	Bifidobacterium longum subsp. Infantis (99.4)
Spirochaetes							
>JK_9-00017	3.42	4.03	3.69	2.33	2.61	3.29	Uncultured Spirochaetes (98.1)
Planctomycetota							
>JK_39-00111	2.31	3.22	2.13	1.63	3.16	2.32	Uncultured Planctomycete (99.4)

Table 3.10. Mean relative abundance of the main bacterial OTUs identified in piglets. Abundance is presented as a percentage (%) of the total number of analyzed reads per sample.

	D18		PWD7		PWD 14		PWD 28		
OTU	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**	**CON**	**SUP**	**Closest Taxon (id%)**
Firmicutes									
>JK_30-00008	1.72	1.15	2.22	0.57	13.64	14.11	5.29	3.33	Lactobacillus amylovorus (99.8)
>JK_43-00101	4.66	4.53	0.74	0.70	0.31	0.37	0.36	0.77	Uncultured Firmicutes (99.8)
>JK_28-03089	0.00	0.00	1.26	1.61	3.30	3.83	1.09	0.96	Uncultured Firmicutes (99.2)
>JK_48-03627	1.10	0.21	0.39	2.20	3.09	1.91	0.13	0.05	Ligilactobacillus salivarius (99.6)
>JK_37-01274	3.40	2.12	0.28	0.05	0.40	0.36	0.24	0.28	Lactobacillus mucosae (99.5)
>JK_16-07146	0.07	0.18	0.03	0.15	0.20	0.46	3.46	1.58	Megasphaera elsdenii (99.8)
Proteobacteria									
>JK_137-00038	0.05	0.05	11.04	5.76	0.01	0.00	0.00	0.00	Uncultured Proteobacterium (99.1)
>JK_134-00239	0.01	0.01	2.96	4.19	0.00	0.00	0.00	0.00	Moraxella osloensis (99.4)
>JK_125-00735	0.01	3.86	0.01	0.01	0.00	0.00	0.00	0.00	Comamonas kerstersii (99.4)
Bacteroidetes									
>JK_38-00035	14.72	16.64	0.04	0.04	0.01	0.00	0.00	0.00	Bacteroides fragilis (99.6)
Actinobacteria									
>JK_134-00684	0.02	0.04	4.08	3.63	0.00	0.00	0.00	0.00	Uncultured Actinobacterium (100)
Planctomycetota									
>JK_39-00111	2.69	3.24	1.36	0.76	0.70	0.83	0.01	0.46	Uncultured Planctomycete (99.4)
Unknown organisms									
>JK_45-00042	27.26	20.05	0.25	0.28	0.62	0.48	0.12	0.51	Uncultured bacterium (99.8)
>JK_51-00117	0.01	0.01	0.62	1.61	3.95	2.97	7.66	5.32	Uncultured bacterium (99.8)
>JK_-42	0.52	0.25	0.57	1.97	8.98	5.78	1.05	0.59	Uncultured bacterium (98.9)
>JK_15-00714	1.50	0.25	5.87	4.67	2.94	1.84	1.35	1.26	Uncultured bacterium (99.8)

Chapter 4

4.0 General Discussion and Conclusions

Assessment of the inclusion of yeast postbiotics on the reproductive performance of sows, growth performance of her offspring to market, sow fecal microbiome, and piglet microbial succession was the focus of this thesis. It was hypothesized that yeast postbiotics would create shifts in the sow fecal microbiome, thus shifting her offspring fecal microbiome via microbial succession to improve subsequent post wean performance (Chapter 3) and create the potential for lowering protein and energy concentrations in late finishing diets (Chapter 2). Modern genetic selection has focused on selecting for hyper prolific sows with increasingly lean, efficient, and fast-growing offspring. The improvement in number of pigs born per sow has placed an ever increasing metabolic and nutritional demand on the sow to support a greater number of highly efficient progeny (Kim et al., 2013; Tokach et al., 2019). The inadequacy in modern sow longevity being observed is a reflection of the metabolic and physiological demands correlated with gestating and suckling a large number of offspring (Engblom et al., 2008; Kim et al., 2013; Tokach et al., 2019). Piglets with decreased viability due to an inability of the sow to support piglets from increased litter sizes are also less prepared to navigate the stress associated with weaning including dietary, environmental, and social changes (Campbell et al., 2013; Moeser et al., 2017). Thus devising nutritional strategies to assist modern hyper prolific sows in rearing large litter sizes is essential to sustain economic profitability in the swine industry.

Yeast fermentation products (i.e. yeast postbiotics) are a relatively new, promising class of feed additives which have emerged. These feed additives are being investigated for their ability to induce changes in the gut microbiome and therefore elucidate positive

changes on the host. The success of utilization of these products to impact the sow microbiome and litter performance has been observed to vary on a case-by-case scenario (Veum et al., 1995; Kim et al., 2008; Callens et al., 2015; Wang et al., 2018; Costa et al., 2019; Shao et al., 2020; Uryu et al., 2020). In chapter 3 the observed effects of yeast postbiotic on litter performance were minimal. This inefficacy may be due to an inadequacy in supplementation level, supplementation time, or inadequate antioxidant intake levels for the animal (Farrugia and Balzan, 2012). Interestingly the uplift in growth performance of finishing animals noted in Chapter 2 may provide insight into an opportunity for application of yeast postbiotics. With lower supplementation levels potentially underserving the large stress events of farrowing, lactation, and weaning in sows and young piglets, it may still provide enough stress relief to assist in heat stress during summer noted in Chapter 2 even during times of lower dietary nutrient concentrations. This variability in the observation of performance effects supports previous research; however, the potential benefits of yeast postbiotics warrants more investigation in order to realize their upside. Reducing sickness and improving oxidative status of the sow, weaning piglet, and finishing pig during farrowing, weaning, and times of heat stress may allow for more energy to be utilized for growth, performance, and longevity.

Although much research has been done in recent years in an attempt to capture the meaning of specific shifts in swine gut bacteria due to specific physiological events (e.g. weaning or farrowing), this information remains difficult to characterize. Intriguingly, grouping individual fecal sampled piglets into 3 groups based on average daily gain (ADG) in the nursery period (high ADG, middle ADG, and low ADG) revealed some

differences in certain bacterial families, although this was not tested statistically. Piglets categorized in the high ADG category had a lower relative abundance of unclassified bacteria than piglets in the low ADG category (15% vs 23%). Piglets in the high ADG category had marginally higher relative abundance of the family *Yersiniaceae* than piglets in the low ADG category (5% vs 0%). A greater proportion of piglets in the high ADG category belonged to piglets from the second farrowing group. Overall, all piglets from the second farrowing group had a greater nursery ADG than all piglets from the first farrowing group (0.83 vs 0.79). Slight differences in nursery fecal bacterial families were noted between the first and second farrowing groups respectively for unclassified bacteria (20% vs 16%), *Ruminococcaceae* (16% vs 10%), *Yersiniaceae* (0% vs 5%), *Moraxellaceae* (0% vs 3%), and *Prevotellaceae* (11% vs 7%). In chapter 3 the negative implications associated with *Yersiniaceae* and *Moraxellaceae* in the fecal microbiome were discussed; however, these populations were present in the higher ADG farrowing group and absent in the lower performing farrowing group. This remains an area of further investigation. Similarly, individual fecal sampled sows grouped into 3 groups based on number of piglets born alive (BA) showed some small bacterial family differences based on their performance grouping. Sows in the high BA category had a lower relative abundance of *Lactobacillaceae* compared to the low BA category (6% vs 14%) as well as a slightly lower relative abundance of *Bifidobacteriaceae* (1% vs 4%). Sows in the low BA category had a marginally lower relative abundance of *Peptostreptococcaceae* compared to sows in the high BA category (16% vs 19%). A similar trend as discussed above in piglet nursery performance was observed for sow BA performance with more sows from the second farrowing group falling into the high BA

category compared to all sows in the first farrowing group. There were some bacterial family differences between sow groups as well with sows from the first farrowing group having a higher relative abundance of *Lactobacillaceae* (12% vs 7%) and *Bifidobacteriaceae* (4% vs 1%) and a lower relative abundance of *Peptostrepococcaceae* (14% vs 19%) compared to sows from the second farrowing group. These differences in fecal bacterial family populations in sows compared to their performance is significant as the *Lactobacillaceae* and *Bifidobacteriaceae* families are widely regarded to contain beneficial species of bacteria for the host. This lack of understanding how sow and piglet performance relate to the fecal microbiome simply highlights the conclusion that more investigation into the gut bacterial communities of swine is needed. This is especially important as more ties are made between the gut microbiome and health and growth.

The study objective was to observe the impacts of including a yeast fermentation postbiotic in gestation and lactation diets on sow reproductive performance, sow fecal microbiome composition, offspring performance through the nursery, and offspring fecal microbiome composition. It was hypothesized that the inclusion of the yeast postbiotic would influence the sow microbiome composition and offspring microbial communities and ultimately improve offspring performance during the suckling and the nursery period. Little is known about how different sow gut microbial compositions translate via microbial succession to a piglet's gut microbiome during the suckling period and into the post-wean period. This study attempted to characterize specific sow or piglet gut communities which correlate to improved growth performance in their offspring or gut communities associated with microbial succession. Yeast postbiotics, as applied in this study, has no impact on the sow microbiome, microbial succession in piglets, or offspring

nursery growth performance. In conclusion, yeast postbiotics may have potential for application in swine late finishing diets.

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