EFFECTS OF FEEDING SOLUBLE FIBER (DEXTRIN) TO PIGS PRE- AND POST-WEANING ON GROWTH PERFORMANCE, INTESTINAL MICROBIOME, VOLATILE FATTY ACID (VFA) PRODUCTION, INTESTINAL MORPHOLOGY, AND GENE EXPRESSION

by

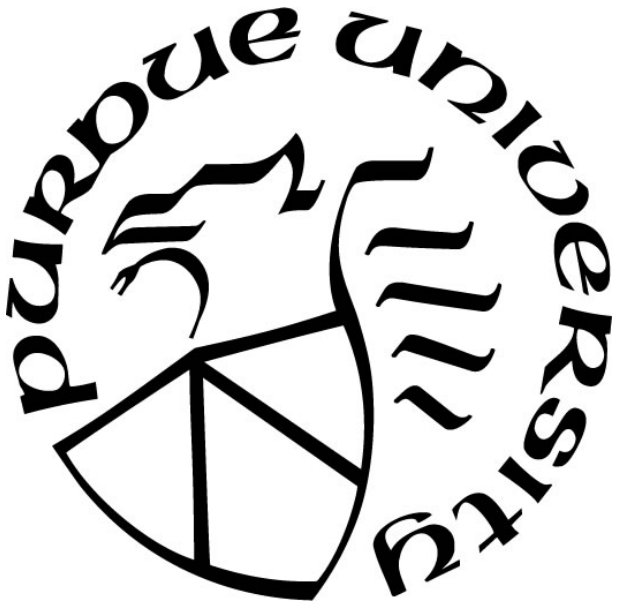
Clay Chastain

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science



Department of Animal Sciences

West Lafayette, Indiana

August 2019

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. J. Scott Radcliffe, Chair

Department of Animal Sciences

Dr. Brian T. Richert

Department of Animal Sciences

Dr. Allan P. Schinckel

Department of Animal Sciences

Approved by:

Dr. Ryan A. Cabot

Head of the Graduate Program

To my father Jeffrey Chastain for initiating my interest to pursue a career within the swine industry. Thank you for your dedication and love as a father and mentor.

ACKNOWLEDGMENTS

I would like to take this opportunity to show my gratitude towards everyone that has helped me the last few years from beginning my college education to finishing my time in graduate school. I firstly would like to thank my advisor, Dr. Radcliffe, for taking me under his wing for the last two years. Though I had some swine experience, I had absolutely no lab experience, but he decided to take a risk on me anyway. I cannot count the number of times that I have been in his office asking for help or guidance on whatever it is I am writing or working on in the lab. No matter how busy this man was, he was always available to help me whenever I needed it (which was more often than I care to admit). I would also like to thank Dr. Richert for making himself available to me whenever I needed his help, from being at the farm at the crack of dawn to help weigh pigs, or staying with me at my desk till around midnight to help me allot my pigs for the beginning of my study. Not to mention the fact that between Dr. Radcliffe and Dr. Richert, enough money was spent on me at restaurants for lab meetings to probably cover a semester's worth of my tuition.

I would also like to take this time to thank my other lab group members for helping me in the mornings with my study, and I would especially like to thank Kayla Mills and Morgan Thayer. Kayla and Morgan have unofficially earned a seat on my "Honorary Graduate Committee" for taking so much time to help me through grad school and walk me through a wide variety of multiple steps, helping me understand how this entire system works.

I would like to thank my family and friends for encouraging me to continually pursue my education in this field and supporting and loving me the entire way.

And lastly, I would like to thank God for providing me with these many opportunities and surrounding me with people who love and help me along the way.

In South Africa, there is a word of Nguni descent that is commonly used. That word is "Ubuntu", which roughly translates to "We are who we are because of others" and no other word nor phrase could better describe how I have made it to where I stand today.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Chastain, Clayton, M.S.
Institution: Purdue University
Degree Received: August 2019
Title: Effects of Feeding Soluble Fiber (Dextrin) to Pigs Pre- and Post-Weaning on Growth Performance, Intestinal Microbiome, Volatile Fatty Acid Production, Intestinal Morphology, and Gene Expression.
Major Professor: John S. Radcliffe

Forty barrows were used in a 35d experiment to evaluate the effects of supplemental soluble fiber (dextrin) pre- and post-weaning on growth performance, intestinal microbiome, volatile fatty acid (VFA) production, intestinal morphology, and gene expression. Pigs were blocked by litter and BW, and randomly allotted to treatments in a 2x2 factorial design with or without fiber pre-weaning and with or without fiber post-weaning. Dextrin was administered orally through a syringe, after being suspended in chocolate milk from 14d prior to weaning through 3d post-weaning, after which it was included in the diet at 1%. At weaning, pigs were group housed by treatment and allowed ad libitum access to a common starter diet. On d4 post-weaning, pigs were moved to individual pens and fed diets with or without 1% fiber. Weights and feed intake were recorded 14 and 3d prior to weaning, and on d0, 4, 11, and 21 post-weaning. On d0 and d21 post-weaning, pigs were euthanized for collection of tissues and intestinal contents. Ileal, cecal, and colon contents were taken for microbiome analysis, distal large intestine contents were collected for VFA analysis, ileal cross sections were collected for histology, and ileal and cecal mucosal scrapings were collected for intestinal gene expression. Data were analyzed using the GLM procedure of SAS with pig as the experimental unit for growth performance, VFA production, intestinal morphology, and gene expression. Microbiome data were analyzed using Metastats, to find statistical significance between treatments, and then run through R, using the false discovery rate method, to find a multiple test corrections q-value. Growth performance in general was not affected ($P > 0.10$) by treatment with the exception of d11-21 feed efficiency was improved ($P = 0.018$) for pigs receiving supplemental fiber prior to weaning. Pigs that received fiber at any point had increased short chain fatty acid (SCFA) producing bacteria ($q < 0.05$) compared to pigs never receiving fiber. Pigs never receiving fiber

had increased bacteria associated with intestinal inflammation ($q < 0.05$) compared to all other treatment groups. A trend for an interaction ($P = 0.054$) of pre- and post-weaning fiber supplementation was observed for total volatile fatty acid concentration in large intestine contents. An interaction ($P = 0.007$) of pre- and post-weaning treatments was observed on butyrate, with pigs fed fiber only during pre-weaning having the greatest butyrate concentrations. Pigs fed fiber pre-weaning had decreased isobutyrate concentrations ($P = 0.050$) and percentages ($P =0.040$) and a trend for decreased isovalerate as a concentration ($P = 0.058$) and percent of total VFAs ($P = 0.051$). Pigs fed fiber post-weaning had increased acetate ($P = 0.047$). An interaction for butyrate percentages was observed with pigs receiving supplemental fiber only prior to weaning having the highest percent of butyrate ($P = 0.029$). An interaction for valerate concentrations ($P = 0.045$) occurred with pigs receiving fiber only prior to weaning having the highest amount of valerate. Valerate as a proportion of total VFAs ($P = 0.038$) was decreased in pigs receiving supplemental fiber post-weaning. Pigs fed fiber prior to weaning tended to have decreased crypt depths ($P = 0.097$) compared to pigs that did not receive fiber prior to weaning. In the ileum there was an interaction ($P = 0.002$) for GLP-2 expression, with pigs receiving supplemental fiber solely before or after weaning having the greatest expression. Occludin expression in the ileum tended to increase with fiber supplementation prior to weaning ($P = 0.086$) but then tended to decrease with fiber supplementation post-weaning ($P = 0.053$) In the cecum, there was an interaction ($P = 0.049$) of pre- and post-weaning fiber supplementation on GLP-2 expression. Pigs fed supplemental fiber at any point had increased GLP-2 expression, but pigs that had fiber only after weaning had the greatest GLP-2 expression. Cecal HSP-70 expression also increased with fiber supplementation in pigs fed fiber post-weaning ($P = 0.012$). Soluble fiber supplementation caused alterations in the intestinal microbiome, VFA concentrations, the intestinal morphology, and in the expression of different intestinal genes.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Weaning is a stressful event early in a pig's life. Many changes occur simultaneously which can make the transition challenging for the animal. During this time there are 3 types of stress that affect the pig: environmental, social, and nutritional (Aherne et al., 1993). Nutritional stress is caused by switching from milk to a dry feed diet. During this switch, pigs may refuse to eat for upwards of 15 hours and then hunger eventually urges them to eat a large amount of feed at once (Aherne et al., 1993). This diet transition as well as unusual eating habits can cause a lot of stress on the gastrointestinal tract, which may impact gut development throughout their lifetime (Moeser et al., 2007a; Moeser et al., 2007b; Campbell et al., 2013). Pigs also face social and environmental stressors that can affect their performance such as changes in housing environment, introduction to new and a greater number of pigs, and loss of maternal dam (Hötzel et al., 2011). All the different stressors around weaning can negatively affect the health and thus growth of the pig. Dietary fiber, when added to the diet, has been known to reduce inflammation and improve gut health in animals (Perry and Ying, 2016; Burkitt et al., 1972; Krishnamurthy et al., 2012; Galisteo et al., 2008; Superchi et al., 2017). The purpose of this review is to further analyze the effects of fiber on the gastrointestinal tract and how it can impact health and immune system development in weanling pigs post-weaning.

1.2 Effects of Weaning Stress

1.2.1 Nutritional Weaning Stress

In nature, pigs are weaned at approximately 3 months of age (Newberry and Wood-Gush, 1988). The average weaning age of pigs in the United States in 2019 was 20.79 days of age.

(PigCHAMP, 2019) This early weaning will ultimately increase growth performance rate but causes health issues in the pig along the way, and it may slow growth performance for a short time after weaning (Pohl et al., 2017). At 5 weeks of age, the digestive system of the pig is more adept at handling cereal based solid food, so weaning before this time, and introducing a large amount of cereal based solid food at once, can cause diarrhea in the pig. The erratic food intake combined with incomplete digestion can cause slowed growth for upwards of 14 days post-weaning (Aherne et al., 1993). Low feed intake is one of many physiological changes that occurs in the pig at weaning. Changes in intestine size and functionality, absorptive capacity, enzyme concentrations, risk of enteric diseases, and gut permeability due to low tight junction expression are all expected to be seen during this time which will be discussed below.

1.2.2 Structural Changes in the Small Intestine Around Weaning

The size of the small intestine also changes during the post-weaning period. During this low intake period, when the pig is deficient in energy and many nutrients, the pigs' body highly prioritizes the growth of intestinal tissue over growth of other organs and tissues (Fenton et al., 1985). This finding was confirmed by Seve et al. (1986) who reported that the digestive organ growth was prioritized higher than the growth of other tissues in the pig's body. The small intestine grows relative to the rest of the body from 28-36 days before birth up until parturition (Marrable, 1971). This trend continues through one-week post-parturition, when the small intestine starts to grow isometric in relation to the rest of the body through six weeks of suckling (Cranwell, 1995). Once weaning occurs, the small intestine growth rate increases. Cera et al. (1998) conducted a study that reported an increase of 84-98% in the weight of the small intestine, when compared to the relative body weight, from birth to 21 days later at weaning. Tarvid et al. (1994) also discovered that intestine weight per intestine length (g/m) increased

280% while length per total body weight decreased fourfold during a period of 30 days from birth through weaning at day 30. This indicates that the intestine becomes thicker during this period at a faster rate than it grows in length. Shields et al. (1980) discovered a similar finding seeing the length of the small intestine per unit of bodyweight decreases after weaning in pigs 8-10 weeks of age (Figure 1.1).

So, while weight of the small intestine increases after weaning, the length does not increase at the same relative rate. For the weight to increase while the length decreases in relation to body weight, the diameter and capacity of the small intestine must increase. A study done by Vodovar et al. (1964) confirms that diameter increases by 3.5-fold and capacity increases by 43-fold starting from birth until 8-10 weeks of age.

1.2.3 Weaning Effect on Intestinal Villi and Crypts

The inner lumen of the small intestine is lined with small finger-like projections protruding into the lumen called villi. At the base of each of these villi are crypts of Lieberkühn, which are tubular intestinal glands (Cranwell, 1995). These villi are primarily lined with enterocytes and goblet cells. The enterocytes will migrate from the crypt to the villus tip where they are sloughed off. During this time, they will mature both functionally and structurally, and the microvilli will rapidly elongate. (Cranwell, 1995). Villus height and crypt depth are commonly used as indicators of absorptive capacity in the small intestine. A shortened villus height and increased crypt depth indicate a reduction in intestinal nutrient absorptive capacity (Wijtten et al., 2011). The jejunum has longer intestinal villi than any other segment of the small intestine (Wiyaporn et al., 2013), and partly due to this, the jejunum sees a greater amount of morphological changes in villi structure than the duodenum or ileum (Komai and Kimura, 1979). From birth to day 3 of age, the villi can double in length from 200-300 μm to >500 μm

(Skrzypek et al., 2010). After day 3, the villi will shorten in length while simultaneously increasing in width causing them to become more leaf-like in shape (Cera et al., 1988). Some morphological changes seen include a decrease in surface area due to the shortening of the height of the intestinal villi. Cera et al. (1988) reported a 65% reduction in jejunal villus height in pigs weaned at 3 weeks and a 27% reduction in pigs weaned at 5 weeks. The pigs weaned at 5 weeks took 3 days to adapt to the new environment and began to grow again. Whereas the pigs weaned at 3 weeks required at least 7 days to recover from the intestinal stress and required 21 days before the villi began to increase in length again (Cera et al., 1988). Cera et al. (1988) also noticed a reduction in microvilli from day 3 to day 7 post-weaning. These findings are in agreement with other studies that reported villus atrophy during a low feed intake period after weaning (Lalles et al., 2004; Pluske et al., 1996; van Beers-Schreurs et al., 1998). Because of these intestinal changes at weaning, pigs are more susceptible to infection, which can drastically affect feed intake, disease resistance, and growth rate, making the immune system play a large role in gut health and functionality.

1.3 The Digestive Tracts Effect on Swine Immunity

1.3.1 Acquisition of Immunity

Health problems continuously affect the swine industry today and can greatly affect profit of the industry as well as the reproductive capacity of the animals. Development of a strong immune system in the young pig allows them to reach a higher growth potential as they age. Pre-weaning mortality was reported to be 14.55% in 2019(PigCHAMP, 2019), and this is partly due to the fact that pigs have an incompetent immune system at birth and are highly susceptible to pathogenic infection (Gaskins and Kelley, 1995). Pigs have an epitheliochorial placenta that does not allow immunoglobulins to transfer in utero. Therefore, pigs are born without any form of

passive immunity and rely on colostrum to acquire immunoglobulins (Stokes and Bourne, 1989). The immune system has several different roles which are primarily classified under passive and active immunity. Active immunity has many different roles within the immune system, some of which will be discussed below.

1.3.2 Passive Immunity Development

The newborn pig is severely immunosuppressed due to a lack of immunoglobulins (Stokes and Bourne, 1989). Sow's colostrum contains high concentrations of IgG for a short time after parturition which pigs will ingest and be able to absorb, thus gaining some of the sow's passive immunity. The amount of immunity they receive is dictated by the quantity and quality of antibodies they ingest (Holland, 1990). Since the only antibodies newborns receive are from the sow, they only have immunity to antigens in which the sow has already developed memory B cells (Porter, 1986). Although this immunity is vastly important for the pig's development, most of the antigens the pigs will come into contact with will be at the mucosal surfaces of the digestive tract of the pig where IgG is at low levels and not very effective (Gaskins and Kelley, 1995). As the sow ceases to produce colostrum, IgG concentrations in her milk decrease and IgA concentrations in milk increase. This is a signal that the mammary glands are producing milk antibodies instead of secreting immunoglobulins from the serum (Bourne et al., 1978; Poonsuk and Zimmerman, 2017). This IgA inoculation concentrates more at the mucosal layer since IgA is resistant to intestinal breakdown. The IgA concentrations are very high at the mucosal level and can lyse and destroy most viral or bacterial antigens that the animal may consume (Porter, 1986). The same concept applies as described earlier, IgA antibodies that the pigs receive will only be sensitive to antigens that the sow has developed memory B cells to. Gut closure begins approximately 24 hours after birth, so it is imperative for the pig to suckle the sow and ingest

colostrum prior to gut closure (Dividich et al., 2005). A study done by Devillers et al. (2011) concluded that pigs that live to weaning typically have an average colostrum intake of 333 ± 14 grams, and pigs that die before weaning ingested less than 277 grams of colostrum. Once gut closure is complete, antibodies will not be absorbed (Weström et al., 1985). Immunoglobulins in milk consumed prior to gut closure will be absorbed in the jejunum and lymphatic vessels. Antibodies then travel through the thoracic duct and mix with intestinal lymph which is then put into circulation. Twenty-four hours post parturition, the pig has similar antibody titers to that of the sow (Holland, 1990). Within 6 hours after nursing begins, immunoglobulin concentrations in milk drop to 50% of their original concentration. Colostrum secretion ceases at approximately 72 hours post parturition (Gaskins and Kelley, 1995; Poonsuk and Zimmerman, 2017). So earlier born pigs have more access to antibody rich colostrum since parturition can last 4-6 hours (Hendrix et al., 1978). Gut closure is also thought to be caused by certain factors in colostrum (Svendsen et al., 1990), but the factors that cause gut closure are currently unknown. Some studies such as ones done by Arvola et al. (1992,1993) report that antigens present in cow's milk when fed to rats delayed gut closure, whereas Jensen et al. (2001) reported that pigs fed both bovine colostrum and milk replacer had a faster gut closure compared to pigs fed sow colostrum. However, while there is debate on what causes gut closure, it can be concluded that colostrum consumption immediately after birth is imperative for pigs to develop a strong passive immune system.

1.3.3 Mucosal Immunity

The intestinal epithelium has a vast surface area to maximize nutrient absorption, but in doing so, also gives rise to increased pathogen accessibility to the epithelium for attachment and absorption. To counteract this, the intestinal tract has developed a system that utilizes immune

factors, both innate and acquired, to prevent attachment of pathogens to intestinal epithelium (Gaskins and Kelley, 1995). Certain recognition systems need to be in place so that the body can distinguish nutrients from antigens. If the body is unable to do this effectively, food allergies may develop (Cordle et al., 2004; Bailey et al., 2005). Recognition systems can either be innate or active. The innate recognition systems work primarily in receptors that have the ability to detect foreign macromolecules in the body to initiate other defense mechanisms (Bailey et al., 2005). These receptors, called pattern-recognition receptors, are capable of recognizing and binding macromolecules from a multitude of pathogenic organisms. The body is even able to recognize harmful macromolecules that it has never been exposed to. This is possible since germ-line genes encode the specificity of these receptor molecules. Germ-line genes are genes passed down to each continuing generation (Chong and Whitelaw, 2004). This allows offspring to have recognition receptors at a young age without being inoculated with certain pathogens (Bailey et al., 2005).

Unlike innate recognition systems, adaptive recognition systems do not inherit receptor specificities. While B and T cell genes are passed down from the previous generation, their specificities are accumulated in lymphocytes. These lymphocytes generate specific targeting sequences by rearranging genes and through hypermutation (Bailey et al., 2005). As more specificities are generated on receptor molecules, the recognition systems become more complex so that they can handle a multitude of different infectious pathogens (Bailey et al., 2005). These systems develop during the beginning stages of life for a pig due to a rapidly increased exposure to unfamiliar antigens. This exposure happens primarily at birth and at weaning, but also between these stages as well (Bailey et al., 2005). At birth the exposure of antigens will be due to the new environment whereas at weaning many of the new antigens that they will be exposed to

will be due to the change in diet. Post-parturition, much of the protection from antigens is due to colostrum. A polypeptide found in sow's milk, TGF- β, is found in high concentrations in colostrum and decreases with the amount of days lactating (Xu et al., 2004). The neonate is able to absorb TGF-β which stimulates IgA production within the small intestine and upregulates non-inflammatory defense mechanisms to help improve the developing immune system of the neonate (Brenmoehl et al., 2018). This switch to IgA production along with other factors may contribute to the suppression of the active immune system development (Bailey et al., 2005). At weaning, upon the transition to eating solid foods, the developed active immune system may prove to be troublesome by potentially activating an immune response to fed proteins that the body has not encountered before (Bailey et al., 2005). The mucosal immune system is also dependent on microbial colonization as it appears to be both antigen-specific and antigen-non-specific in nature (Butler et al., 2000). The microbial population utilizes its antigen-non-specific products to be able to ligate the pattern recognition receptors which isnecessary in developing an efficient mucosal immune system (Butler et al., 2000; Bailey et al., 2005).

The role of the mucosal immune system at weaning can be summarized in two main objectives: To recognize and activate the immune system to potential antigens and to develop a certain degree of tolerance to microflora and food proteins that are ingested (Bailey et al., 2005). Failure of the mucosal immune system to perform these tasks results in decreased disease resistance and/or severe allergic reactions to certain food types.

1.3.4 Intestinal Permeability and Cytokine Involvement in the Small Intestine

After birth, the intestines grow rapidly which is coupled with decreased intestinal permeability of tight junctions. This function is essential to prevent the immune system from being introduced to novel antigens in order to inoculate the gastrointestinal tract with colonizing

microbiota and to prevent adverse reactions to specific food allergens, which can cause intestinal distress and inflammation (Moeser et al., 2017). Certain immune factors boost the immune system, such as IgA in colostrum, while others suppress the immune system and allow for microbiota to colonize. Anti-inflammatory peptides and cytokines within the colostrum aide in neonatal immunity by decreasing intestinal permeability to pathogens (Newburg and Walker, 2007). Neonatal pigs have a low titer of pro-inflammatory lymphocytes at birth which also helps in decreasing intestinal permeability (Nguyen et al., 2010).

The early immune system of pigs has lowered expression of T_h1 and upregulated expression of T_h2 cytokines (Beverly, 1997). This allows microbial colonization of the gut since T_h1 cells cause hypersensitivity while T_h2 cause the secretion on B cell antibodies (Romagnani, 1992). In addition, the neonatal system has an increased responsiveness to cytokines such as IL-4 while reduced responsiveness to other cytokines such as IL-12 (Beverly, 1997). Cytokine IL-4 works through a positive feedback system to recruit naïve T_h cells and induce them to differentiate into T_h2 cells. These T_h2 cells then produce more IL-4 to continue the positive feedback (Choi, 1998). Cytokine IL-12 responsiveness is suppressed due to this cytokine upregulating T_h1 responses to induce other cytokines such as IFN-γ which can cause increased hypersensitive reactions and NK cell activity (Sun, 2015). Garcia et al. (2000) reported that neonatal pigs have a shorter CDR3 region on T cell receptors, which decreases the binding ability of molecules to their respective antigens thus decreasing the effectiveness of T cells. As pigs begin to age, their immune system becomes more functional as their lymphocytes begin to develop and become more active throughout the body (Butler et al., 2000, 2009; Butler and Wertz, 2012), which usually occurs around 6 weeks of age (Blikslager et al., 1997). As these lymphocytes become more functional, around 6 weeks of age, they will have the ability to

infiltrate the small intestine. The increased titer of lymphocytes are able to interact with multiple antigens, thus familiarizing the body with self and non-self-antigens. This process helps the body avoid allergic reactions, identify and destroy pathogenic antigens, and allows microbiota to colonize the intestines, (Martin et al., 2010; Wu and Wu, 2012). All of this demonstrates how the neonate as well as the sow's milk contribute to the function and efficiency of the neonate's immune system. A disruption in any of these processes may result in significant immune system disfunction throughout the animal's lifetime (Moeser et al., 2017).

Intestinal permeability decreases after birth but increases following weaning due to multiple stressors around the time of weaning (Aherne et al., 1993; Pohl et al., 2017). This can be measured by transepithelial electrical resistance which decreases in pigs during and after weaning (Pohl et al., 2017). Intestinal permeability peaks 24 hours after weaning and then takes 2 weeks to return to normal (Moeser et al., 2007a). During this time of increased intestinal permeability, molecules are able to pass through the intestinal lining easier than normal, which can cause inflammation leading to intestinal distress (Moeser et al., 2007a; Hu et al., 2013; Pohl et al., 2017). This inflammation is caused by the release of pro-inflammatory cytokines and activation of the intestinal immune system (Hu et al., 2013).

Mast cells have also been shown to play a role in intestinal permeability in pigs (Moeser et al., 2007b; Moeser et al., 2017; Pohl et al., 2017). Mast cells are activated and then typically used for defense mechanisms throughout the body, especially in gastrointestinal disorders such as irritable bowel disease in humans (Boeckxstaens, 2015). Pigs have been reported to have increased intestinal mast cell activation within the first 24 hours after weaning, with pigs weaned earlier having an even greater amount of mast cell activation (Moeser et al., 2007b). This increased mast cell activation at weaning causes hyperplasia of intestinal mast cells into

adulthood (Pohl et al., 2017). Mast cells were shown to play a role in intestinal permeability through the use of the drug sodium cromolyn, which acts as a mast cell stabilizer in vivo. Pigs given this drug prior to weaning showed a resistance to weaning associated increases in intestinal permeability (Moeser et al., 2007b). These studies provide enough evidence to conclude that mast cells play an important role in intestinal permeability in weaned pigs.

The immune system can greatly affect feed intake, disease resistance, and growth rate. In order to try and boost the immune system around the time of weaning, when pigs tend to be immunosuppressed, researchers have experimented with adding fiber to the diet in hopes that it acts as a prebiotic to stimulate positive microflora growth and ameliorate the negative effects that occur around the time of weaning.

1.4 Fiber

1.4.1 The Importance of Fiber

Research over the last 60 years has documented various health benefits of feeding fiber (Burkitt, 1952; Cleave, 1968; Burkitt et al., 1972; Trowell, 1972, 1973). The type of fiber can affect the results it has on the body since different fiber sources have varying functional properties and react differently in the body depending on how each source was processed prior to ingestion (Bosaeus, 2004). Fiber is an indigestible carbohydrate usually found in plant cell walls (Brownlee, 2009). This is how dietary fiber is generally described, but the Codex Committee on Nutrition and Foods for Special Dietary Uses (2009) wrote a definition that is now more generally accepted internationally, it states “Dietary fiber means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans and belong to the following three categories: (1) Edible carbohydrate polymers naturally occurring in the food as consumed, (2) Carbohydrate polymers, which have

been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, (3) Synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities" (Codex Alimentarius Commission, 2009). One way to classify fiber is based on solubility: soluble and insoluble dietary fiber (Brennan, 2005). Based on these classifications and definitions, scientists have narrowed it down to classify fiber by any of the following characteristics: Source, Digestion Resistance, Chemical properties, and Physiological effects (Perry and Ying, 2016). Due to differences in chemical structures of fibers, they tend to have various physiological effects (Anderson et al., 1990; Cui et al., 2009, 2013). Some of these effects are observable by certain soluble dietary fibers having an association with a decrease in blood cholesterol concentration as well as a decrease in glucose absorption throughout the gastrointestinal tract in humans (Angioloni and Collar, 2011). Insoluble dietary fibers have generally been associated with water absorption (Angioloni and Collar, 2011). Fiber is also able to affect the rate of starch degradation in vitro which subsequently decreases the rate of glucose absorption (Oh et al., 2014). Dietary fiber does this by trapping starch molecules and limiting the accessibility of water and starch degrading enzymes to the starch molecules (Brennan, 2005). Some other physical properties of dietary fiber affect the gastrointestinal tract such as, the ability to form gels, which can increase the viscosity of the digesta, and the ability to influence the rate of fermentation in the hindgut (Weickert and Pfeiffer, 2008). Due to the expansive property of fiber, inclusion at high levels can increase satiety in the animal, subsequently lowering the amount of nutrients that are ingested (Khoury et al., 2012). Fiber is a rather broad category, so to understand how fiber can affect the

gastrointestinal tract, we much first discuss the different types of fiber that can be fed to the animal.

1.4.2 Types of Fiber

Soluble fibers have been reported to increase total transit time in the small intestine due to the increased viscosity of digesta. However non-viscous soluble dietary fibers have little effect on viscosity and therefore mainly act as a fermentation source for hindgut microbiota (Khoury et al., 2012). Soluble dietary fibers include fibers such as: oligosaccharides, psyllium, galactomannan gums, alginates, pectins, dextrin, and β-glucans (Perry and Ying, 2016). Oligosaccahrides such as fructooligosaccharides, can be found in some plants which include: artichokes, garlic, asparagus, onions, and wheat (Theuwissen and Mensink, 2008). Psyllium is a general name that encompasses many species of the plant genus Plantago and is primarily located in the many forms of seed husks but is present in the seed as well (Theuwissen and Mensink, 2008). Galactomannans have a heightened ability to bind water compared to other soluble dietary fibers, which makes it a great food stabilizer and emulsifier (Wu et al., 2012). Galactomannan gums can be found in guar gum, tara gum, locust bean gum, and fenugreek gum (Wu et al., 2012). Alginates are typically found in plant cell walls of algae, but can also be found in high concentrations in bacterial exopolysaccharides of Pseudomonas aeruginosa (Pawar and Edgar, 2012). Pectin is typically found in plant cell walls, primarily in fruit skins and is a linear polysaccharide (Theuwissen and Mensink, 2008). The β-glucan polysaccharides are linked by β-glycosidic bonds and usually occur in the form of cellulose in plants and are most commonly found in cereal grains, yeast, fungi, and certain bacteria (Theuwissen and Mensink, 2008).

Insoluble dietary fibers consist of cellulose and most hemicelluloses, as well as lignin and resistant starch (Perry and Ying, 2016). Cellulose is the most abundant polysaccharide on earth

and can have anywhere from 100 - >10,000 glucose monomers that are linked together via β- 1,4 linkages (Klemm et al., 2005). Cellulose can be found largely in cereal bran as well as grains, fruit, vegetables, and nuts, while hemicelluloses are more often found in cereal grains (Fuentos-Zaragoza et al., 2010). What separates resistant starch from other starches is its ability to resist hydrolysis to D- glucose while in the presence of amylase during its time in the small intestine (Fuentos-Zaragoza et al., 2010). Resistant starch is most commonly found in whole grains, legumes, rice, and potatoes (Fuentos-Zaragoza et al., 2010). The main factors that contribute to the ability of starch to be resistant to hydrolysis are the physical form of the starch, associations that may occur between the starch molecule and other food particles in the digesta, and how the food was processed and/or cooked (Fuentos-Zaragoza et al., 2010). Lignin appears mainly in the cell wall component in woody products such as outer layers of cereal grains and seed husks and is composed of multiple aromatic alcohol groups (Fuentos-Zaragoza et al., 2010). All these sources of insoluble dietary fibers tend to contribute the same overall effect which is an increase in the bulk of feces and a laxative effect due to a decreased intestinal transit time (Perry and Ying, 2016).

Dietary fiber, both soluble and insoluble, are susceptible to fermentation, but the level of fermentation can vary based on the type of fiber and associated physical properties (Perry and Ying, 2016). The most fermentable sources of fiber tend to be soluble dietary fiber, oligosaccharides, and resistant starches (Vos et al., 2007). The fermentation of these fibers creates short chain fatty acids (SCFAs) most commonly, acetate, propionate, and butyrate that can be used as energy for the body or for the microbes (Jenkins et al., 2002). Depending on the prevalence and concentration of certain microbes, as well as the source of fiber in the digesta, the amounts of the SCFAs produced can vary widely. The production of SCFAs is believed to be

beneficial when fed to rats as it decreases glycogen breakdown and release of glucose from the liver and also decreases the amount of cholesterol in the blood(Galisteo et al., 2008).

Dietary fiber intake can also affect the microbiota in a way that affects insulin resistance and the development of obesity. Bäckhed et al. (2007) reported that obese mice have a different intestinal microbiome than lean mice, and as obese mice began to lose weight, their microbiome shifted to be more similar to that of the lean mice. Bäckhed et al. (2007) also implanted gnotobiotic mice with the intestinal microbiota of the lean and obese mice and observed that mice implanted with the obese microbiota had an increase in fat content when on a diet similar in energy content. A separate study done by Cani et al. (2007) using humans, showed that those being fed a diet high in soluble dietary fiber had a decrease in gram-negative bacteria as well as body weight while those being fed a diet that had a high fat content developed increased levels of gram-negative bacteria. Lipopolysaccharides, a gram negative bacteria, have been reported in pigs to be associated with a reduction in feed intake, a decrease in normal activity, and an elevation in body temperature (Johnson and von Borrell, 1994).These studies listed above indicate that feeding certain dietary fibers may help in altering the microbiota in a way to increase intestinal health, decrease fat deposition, and decrease lipid concentration in the blood.

1.4.3 Effect of Fiber on Microbiota

Partially mentioned above, along with the other effects, fiber is also able to interact with and alter the gut microbiome (Bauer et al., 2006). Fiber acts as a prebiotic source for intestinal microbiota, boosting beneficial bacteria while inhibiting the growth of some pathogenic bacteria. Wang and Gibson (1993) demonstrated this, reporting that fructo-oligosaccharides and inulin when incubated in-vitro with human feces, decreased the pH of the sample and increased short chain fatty acid production which resulted in decreased concentration of *Clostridium perfringens*

and *E. coli.* Rossi et al. (2001) also reported that pigs inoculated with *E. coli* had a reduced presence of *E. coli* when inulin was included in the diet. It is well known that most fibers primarily increase beneficial bacteria while decreasing pathogenic bacteria. The bacteria that are most often able to utilize soluble fibers are bacteroides, enterobacteria, streptococci, and lactobacilli (Hidaka et al., 1986). Bifidobacteria can also utilize certain fibers such as transgalacto-oligosaccharide (Tanaka et al., 1983). The presence of these beneficial bacteria can increase short chain fatty acid production which is known to increase the amount of natural killer cells (Pratt et al., 1986), reduce concentration of cytokines associated with an inflammatory response (Segain et al., 2000), and make epithelial cells less dependent on glutamine (Zhang et al., 1998), which then allows glutamine to be used as an energy source for lymphocytes (Jenkins et al., 1999). Soluble fiber supplementation is also known to decrease pH of digesta which results in an increase in mucin production in the small intestine (Satchithanandam et al., 1990; Bustos-Fernandez et al., 1978). This increase in mucin production is caused by the increased acidity of short chain fatty acid production, so with increased short chain fatty acid production an increase in mucin production is typically observed (Finnie et al., 1995; Meslin et al., 2001).

The immune system is also greatly boosted with the addition of fiber to the diet. Fiber boosts certain beneficial microbiota while reducing pathogenic bacteria, which can then affect the gut-associated lymphoid tissue, GALT, which is very often altered by lactic acid bacteria (Bauer et al., 2006). Many studies have reported boosts in immune system factors with the addition of fiber. Field et al. (1999) fed fiber to dogs and reported an increased concentration of CD8+ T-cells, Peyer's patches, and CD4+ T-cells around the small intestine but most notably in the mesenteric lymph nodes. A study by Lim et al. (1997) reported a similar result feeding rats a diet including pectin that increased the concentration of CD4+ T-cells, an increase in IgG and

IgA immunoglobulins within the blood serum, an increase in IgA immunoglobulins in the cecum, and an alteration in the production of cytokines in the mesenteric lymph nodes. Bifidobacteria, a bacterial genus generally associated with increased gut health, was shown to increase in pigs fed supplemental fiber (Houdijk et al., 2002). Yun et al. (1997) incorporated β-Glucan into mice diets and reported an increase in IgG in the serum as well as an alteration in the production of cytokines. Yamada et al. (1999) also reported increased IgA and IgG in the mesenteric lymph nodes and the spleen when feeding rats either pectin or glucomannan. All these studies suggest that adding fiber to the diet will alter the efficiency and function of the immune system, which may aid in disease prevention in the animal.

1.4.4 Role of Fiber on Intestinal Inflammation

Fiber consumption has also been linked to reduced inflammation in the gut (Grooms et al., 2013). A study performed by King et al. (2007) reported that a high fiber diet, as well as additional supplementation of soluble dietary fiber decreased concentrations of C-reactive protein, CRP, which is known to be a key inflammatory marker, throughout the body. This could be due to increased butyrate production from fermentation of soluble dietary fiber in the cecum and large intestine (Galisteo et al., 2008). Another study, by Ma et al., (2006) reported that when people began eating more dietary fiber, a reduction in CRP levels was recorded. Krishnamurthy et al., (2012) reported decreased inflammation in patients consuming high fiber diets, and this association was even more prevalent if the patient had kidney disease since kidney disease is recognized as a state of high inflammation. Baggio et al. (2019) reported that incubating cells expressing TLR4 with rhamnogalacturonan activated TLR4 which subsequently decreased the transepithelial electrical resistance of the cells, thus decreasing intestinal permeability.

These studies cited above point to results that suggest fiber supplementation can cause such aschanges in microbiota, decreased inflammation, increased insulin sensitivity, and increased energy for the intestinal mucosal cells through increased SCFA production. All these benefits can be attributed to different physical or chemical properties of fiber such as its viscosity, fermentation capacity, and decreased response of glucose. Dietary fiber has also been linked to alterations in lipid synthesis due to changes in enzyme expression and increased amounts of glucose production (Brennan, 2005). However, the source, type, size, and solubility of fiber may cause different metabolic responses.

1.5 Soy Allergy in Pigs

Soybeans have been used throughout the swine industry due to its high concentration of quality amino acids and high availability (Hancock et al., 2000). However, the downside to using soybean meal, especially in the diets of younger pigs, is that it can cause intestinal disturbances and health problems due to the pigs being susceptible to allergenic proteins in soybeans such as β-conglycinin and glycinin (Barratt et al., 1978; Dréau et al., 1994; Li et al., 1990). β-Conglycinin and glycinin proteins are two of the main allergens found in soybeans and are primary storage proteins (Lallés et al., 1999; Ogawa et al., 1995). Glycining and β-conglycinin are capable of traveling through the stomach without degrading, until the proteins makes it to the small intestine where they are absorbed by the gut epithelium which then activates the allergenic response (Astwood et al., 1996; Moreno and Clemente., 2008). Once β-conglycinin is absorbed, it causes the body to release more IgA, IgE, IgG, and IgM immunoglobulins in calves (Dréau et al., 1995). Glycinin absorption in pigs causes increased concentrations of IgA, IL-4, and IL-6 cytokines (Sun et al., 2008). The responses of pigs to β-conglycinin are typically delayed growth, intestinal disruption, and immune system malfunction (Qiao et al., 2003; Hao et al.,

2009), and glycinin has been reported to decrease growth performance and stimulate various immune responses (Sun et al., 2008). Intestinal disruption usually occurs with the consumption of both proteins with pigs having a decreased absorptive capacity due to decreased villus:crypt (Wang et al., 2014).

This allergy can be exacerbated by intestinal inflammation and lack of intestinal barrier function that typically occurs directly after weaning (Moeser et al., 2007a). The intestines act as a barrier to prevent any absorption of antigens that may be present in the digesta, which is held together by tight junction proteins (Madara et al., 1990). However, when inflammation occurs, these tight junctions can break down and be less functional allowing glycinin, β-conglycinin, and other antigens to bypass the intestinal barrier. Food allergies have also been known to increase intestinal permeability, which consequently allows more of the allergen to traverse through the intestinal epithelium. Since this happens due to the presence of an allergen, it is not necessarily the cause of the allergic reaction, only the result. This increased permeability however, causes a self-perpetuating cycle that keeps the allergic reaction from recovering (Heyman, 2005). In a study done by Zhao et al. (2014), porcine epithelial cells treated with 3 mg/mL β-conglycinin had a greater trans-epithelial electrical resistance due to the allergenic response that β-conglycinin causes. A study performed by Wu et al. (2016), reported the the supplementation of glycinin and the supplementation of β-conglycinin both damaged the intestinal mucosa, increased intestinal permeability, and promoted IgA synthesis. Another study done by Hao et al. (2009), gave pigs an oral gavage of either 2 or 6 grams of β-conglycinin. They reported that orally gavaging pigs with β-conglycinin resulted in symptoms of anaphylaxis such as rashes, scratching, diarrhea, and low activity levels. Unexpectantly however, pigs that were sensitized with 2 grams of β-conglycinin had more severe symptoms than pigs sensitized with 6 grams.

This was thought to be due to the pigs receiving 6 grams building up a tolerance faster due to the increased levels (Hao et al., 2009). These pigs also had decreased growth performance and increased IgE concentrations (Hao et al., 2009).

1.6 Conclusions

Weaning presents multiple forms of stressors that may be harmful to pigs such as absorptive capacity fluctuations and reduced gut permeability. These stressors may vary, but dietary fiber has been shown to decrease the severity of the negative effects caused by these stressors. Different types of dietary fibers affect the gastrointestinal tract in different ways, mainly depending on whether it is soluble or insoluble in water, but the plant source of the fiber can affect its effectiveness as well.

Dietary fiber can be fermented in the cecum and large intestine which can help it function as a prebiotic, aiding the growth of beneficial bacteria while inhibiting the growth of some pathogenic bacteria. This can alter the gut microbiome of the pig and increase intestinal health.

While the pig gains passive immunity from its mother, active immunity will not be fully developed at weaning. With the introduction of a new food source, the immune system is exposed to novel antigens that may cause food allergies or intestinal damage. The immune system must utilize its different recognition systems to identify whether substances in the gastrointestinal tract are harmful. β-Conglycinin in soybeans is an example of a protein that can affect the pig's health and growth performance, as it is known to cause food allergies and to increase intestinal permeability. This makes the mucosal immune system important for it to identify any potential antigens to rid them from the body while also tolerating certain microflora and proteins found in feed that are not harmful to the pig.

Adding dietary fiber reduces intestinal permeability, thus limiting the amount of food allergens that can penetrate the wall of the small intestine, preventing an immune response mounted against the allergens. Fiber also has been shown to increase the total energy available in the diet due to fermentation in the cecum releasing short chain fatty acids.

With all this research, little has is known about supplementation of fiber to the pre-weaning pig. Therefore, we hypothesized that the addition of soluble fiber to the pre-weaning and/or post-weaning pig would prevent some of the negative gastrointestinal effects, such as increased intestinal permeability, and decreased absorptive capacity that weaning induces. The overall objective was to prevent these negative effects from occurring, rather than simply trying to speed up the recovery period after post-weaning stress occurs.

1.7 Literature Cited

Aherne, F., M. Hogberg, and H. Jones. 1993. Management and Nutrition of the Newly Weaned Pig. In: Pork Industry Handbook.

Anderson, J. W., D. A. Deakins, T. L. Floore, B. M. Smith, and S. E. Whitis. 1990. Dietary fiber and coronary heart disease. Crit. Rev. Food Sci. Nutr. 29:95–147. doi:10.1080/10408399009527518.

Angioloni, A., and C. Collar. 2011. Physicochemical and nutritional properties of reduced-caloric density high-fibre breads. LWT - Food Sci. Technol. 44:747–758. doi:10.1016/J.LWT.2010.09.008.

Arvola, T., I. Rantala, A. Marttinen, and E. Isolauri. 1992. Early Dietary Antigens Delay the Development of Gut Mucosal Barrier in Preweaning Rats. J. Pediatr. Res. 32:301–305. doi:10.1203/00006450-199209000-00011.

Arvola, T., E. Isolauri, I. Rantala, M. Kaila, H. Majamaa, E. Virtanen, and H. Arvilommi. 1993. Increased in vitro intestinal permeability in suckling rats exposed to cow milk during lactation. J. Pediatr. Gastroenterol. Nutr. 16:294–300.

Astwood, J. D., J. N. Leach, and R. L. Fuchs. 1996. Stability of food allergens to digestion in vitro. Nat. Biotechnol. 14:1269–1273. doi:10.1038/nbt1096-1269.

Bäckhed, F., J. K. Manchester, C. F. Semenkovich, and J. I. Gordon. 2007. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice.

Baggio, C. H., J. Shang, A. M. Nascimento, T. R. Cipriani, M. Stephens, P. von der Weid, and W. MacNaughton. 2019. Dietary fibre improves intestinal epithelial barrier function through TLR4 activation. J. Can. Assoc. Gastroenterol. 2:147–148. doi:10.1093/jcag/gwz006.072.

Bailey, M., K. Haverson, C. Inman, C. Harris, P. Jones, G. Corfield, B. Miller, and C. Stokes. 2005. The development of the mucosal immune system pre- and post-weaning: balancing regulatory and effector function. Proc. Nutr. Soc. 64:451–457. doi:10.1079/PNS2005452.

Barman, N. N., A. T. J. Bianchi, R. J. Zwart, R. Pabst, and H. J. Rothkötter. 1996. Jejunal and ileal Peyer's patches in pigs differ in their postnatal development. Anat. Embryol. (Berl). 195:41–50. doi:10.1007/s004290050023.

Barratt, M. E. J., P. J. Strachan, and P. Porter. 1978. Antibody mechanisms implicated in digestive disturbances following ingestion of soya protein in calves and piglets. Clin. Exp. Immunol. 31:305-312

Bauer, E., B. A. Williams, and M. W. A. Verstegen. 2006. Fermentable carbohydrates: potential dietary modulators of intestinal physiology, microbiology and immunity in pigs. Biol. Grow. Anim. 4:33–63. doi:10.1016/S1877-1823(09)70089-X.

Beverley, P. C. L. 1997. Vaccine immunity. Immunol. Today. 18:413–415. doi:10.1016/S0167-5699(97)01120-1.

Blikslager, A. T., M. C. Roberts, J. M. Rhoads, and R. A. Argenzio. 1997. Is reperfusion injury an important cause of mucosal damage after porcine intestinal ischemia? Surgery. 121:526–534. doi:10.1016/S0039-6060(97)90107-0.

Boeckxstaens, G. 2015. Mast cells and inflammatory bowel disease. Curr. Opin. Pharmacol. 25:45–49. doi:10.1016/J.COPH.2015.11.005.

Bosaeus, I. 2004. Fibre effects on intestinal functions (diarrhoea, constipation and irritable bowel syndrome). Clin. Nutr. Suppl. 1:33–38. doi:10.1016/J.CLNU.2004.09.006.

Bourne, F. J., T. J. Newby, P. Evans, and K. Morgan. 1978. The Immune Requirements of the Newborn Pig and Calf. In: Annales de Recherches Veterinaires. 9th ed. p. 239-244.

Brenmoehl, J., D. Ohde, and E. Wirthgen. 2018. Cytokines in milk and the role of TGF-beta. Best Pract. Res. Clin. Endocrinol. Metab. 32:47–56. doi:10.1016/J.BEEM.2018.01.006.

Brennan, C. S. 2005. Dietary fibre, glycaemic response, and diabetes. Mol. Nutr. Food Res. 49:560–570. doi:10.1002/mnfr.200500025.

Brownlee, I. A. 2011. The physiological roles of dietary fibre. Food Hydrocoll. 25:238–250. doi:10.1016/J.FOODHYD.2009.11.013.

Burkitt, D. P. 1952. Acute abdomens--British and Baganda compared. East Afr. Med. J. 29:189–94.

Burkitt, D. P., A. R. Walker, and N. Painter. 1972. Effect of dietary fibre on stools and transit-times, and its role in the causation of disease. Lancet. 300:1408–1411. doi:10.1016/S0140-6736(72)92974-1.

Bustos-Fernandez, L., I. L. De Paolo, S. Hamamura, E. Gonzalez, D. Celener, M. I. Caldarini, and O. M. Tiscornia. 1978. Does Secretin Influence Rat Colonic Absorption and Secretion? Am. J. Gastroenterol. 70:265–269.

Butler, J. E., K. M. Lager, I. Splichal, D. Francis, I. Kacskovics, M. Sinkora, N. Wertz, J. Sun, Y. Zhao, W. R. Brown, R. DeWald, S. Dierks, S. Muyldermans, J. K. Lunney, P. B. McCray, C. S. Rogers, M. J. Welsh, P. Navarro, F. Klobasa, F. Habe, and J. Ramsoondar. 2009. The piglet as a model for B cell and immune system development. Vet. Immunol. Immunopathol. 128:147–170. doi:10.1016/J.VETIMM.2008.10.321.

Butler, J. E., J. Sun, P. Weber, P. Navarro, and D. Francis. 2000. Antibody repertoire development in fetal and newborn piglets, III. Colonization of the gastrointestinal tract selectively diversifies the preimmune repertoire in mucosal lymphoid tissues. Immunology. 100:119–130. doi:10.1046/j.1365-2567.2000.00013.x.

Butler, J. E., P. Weber, M. Sinkora, J. Sun, S. J. Ford, and R. K. Christenson. 2000. The Journal of Immunology. J. Immunol. 152:632–641. doi:10.4049/jimmunol.165.12.6999.

Butler, J. E., and N. Wertz. 2012. The Porcine Antibody Repertoire: Variations on the Textbook Theme. Front. Immunol. 3:153. doi:10.3389/fimmu.2012.00153.

Cani, P. D., J. Amar, M. A. Iglesias, M. Poggi, C. Knauf, D. Bastelica, A. M. Neyrinck, F. Fava, K. M. Tuohy, C. Chabo, T. Sulpice, B. Chamontin, G. R. Gibson, L. Casteilla, N. M. Delzenne, and M. C. Alessi. 2007. Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. Diabetes. 56:1761–1772. doi:10.2337/db06-1491.P.D.C.

Cera, K. R., D. C. Mahan, and R. F. Cross. 1988. Effect of Age , Weaning, and Postweaning Diet on Small Intestinal Growth and Jejunal Morphology in Young Swine 1 ' 2. 574–584.

Choi, P., and H. Reiser. 1998. IL-4: role in disease and regulation of production. Clin. Exp. Immunol. 113:317–9. doi:10.1046/J.1365-2249.1998.00690.X.

Chong, S., and E. Whitelaw. 2004. Epigenetic germline inheritance. Curr. Opin. Genet. Dev. 14:692–696. doi:10.1016/J.GDE.2004.09.001.

Cleave, T. L. 1968. SUCROSE INTAKE AND CORONARY HEART-DISEASE. Lancet. 292:1187. doi:10.1016/S0140-6736(68)91659-0.

Codex Alimentarius Commission. 2009. Guidelines on Nutritional Labeling. Report of the 31st Session of the Codex Committee on Nutrition and Foods for Special Dietary Uses, Dusseldorf, Germany

Cordle, C. T. 2004. Soy Protein Allergy: Incidence and Relative Severity. J. Nutr. 134:1213S–1219S. doi:10.1093/jn/134.5.1213S.

Cranwell, P. D. 1995. Development of the Neonatal Gut and Enzyme Systems. In: M. A. Varley, editor. The Neontal Pig: Development and Survival. CABi. p. 99–154.

Cui, S. W., and K. T. Roberts. 2009. Dietary Fiber: Fulfilling the Promise of Added-Value Formulations. Mod. Biopolym. Sci. 399–448. doi:10.1016/B978-0-12-374195-0.00013-6.

Cui, S. W., Y. Wu, and H. Ding. 2013. The range of dietary fibre ingredients and a comparison of their technical functionality. Fibre-Rich and Wholegrain Foods. 96–119. doi:10.1533/9780857095787.1.96.

Devillers, N., J. Le Dividich, and A. Prunier. 2011. Influence of colostrum intake on piglet survival and immunity. Animal. 5:1605–1612. doi:10.1017/S175173111100067X.

Dividich, J. LE, J. A. Rooke, and P. Herpin. 2005. Nutritional and immunological importance of colostrum for the new-born pig. J. Agric. Sci. 143:469–485. doi:10.1017/S0021859605005642.

Dréau, D., J. P. Lallès, V. Philouze-Romé, R. Toullec, and H. Salmon. 1994. Local and systemic immune responses to soybean protein ingestion in early-weaned pigs. J. Anim. Sci. 72:2090–2098. doi:10.2527/1994.7282090x.

Dréau, D., J. P. Lallès, H. Salmon, and R. Toullec. 1995. IgM, IgA, IgG1 and IgG2 specific responses in blood and gut secretion of calves fed soyabean products. Vet. Immunol. Immunopathol. 47:57–67. doi:10.1016/0165-2427(94)05375-3.

Finnie, I. A., A. D. Dwarakanath, B. A. Taylor, and J. M. Rhodes. 1995. Colonic mucin synthesis is increased by sodium butyrate. Gut. 36:93–99. doi:10.1136/gut.36.1.93.

Fenton, J. P., K. L. Roehrig, D. C. Mahan, and J. R. Corley. 1985. Effect of Swine Weaning Age on Body Fat and Lipogenic Activity in Liver and Adipose Tissue. J. Anim. Sci. 60:190–199. doi:10.2527/jas1985.601190x.

Field, C. J., M. I. McBurney, S. Massimino, M. G. Hayek, and G. D. Sunvold. 1999. The fermentable fiber content of the diet alters the function and composition of canine gut associated lymphoid tissue. Vet. Immunol. Immunopathol. 72:325–341. doi:10.1016/S0165-2427(99)00148-8.

Fuentes-Zaragoza, E., M. J. Riquelme-Navarrete, E. Sánchez-Zapata, and J. A. Pérez-Álvarez. 2010. Resistant starch as functional ingredient: A review. Food Res. Int. 43:931–942. doi:10.1016/J.FOODRES.2010.02.004.

Galisteo, M., J. Duarte, and A. Zarzuelo. 2008. Effects of dietary fibers on disturbances clustered in the metabolic syndrome. J. Nutr. Biochem. 19:71–84. doi:10.1016/J.JNUTBIO.2007.02.009.

Garcia, A. M., S. A. Fadel, S. Cao, and M. Sarzotti. 2000. T Cell Immunity in Neonates. Immunol. Res. 22:177–190. doi:10.1385/IR:22:2-3:177.

Gaskins, H. R., and K. W. Kelley. 1995. Immunology and Neonatal Mortality. In: M. A. Varley, editor. The Neontal Pig: Development and Survival. CABi. p. 39–55.

Grooms, K. N., M. J. Ommerborn, D. Q. Pham, L. Djoussé, and C. R. Clark. 2013. Dietary Fiber Intake and Cardiometabolic Risks among US Adults, NHANES 1999-2010. Am. J. Med. 126:1059–1067.e4. doi:10.1016/J.AMJMED.2013.07.023.

Hancock, J. D., H. Cao, I. H. Kim, and D. F. Li. 2000. Effects of processing technologies and genetic modifications on nutritional value of full-fat soybeans in pigs. In: Asian-Australasian Journal of Animal Sciences. Vol. 13. Asian-Australasian Association of Animal Production Societies. p. 356–375.

Hao, Y., Z. Zhan, P. Guo, X. Piao, and D. Li. 2009. Soybean β-conglycinin-induced gut hypersensitivity reaction in a piglet model. Arch. Anim. Nutr. 63:188–202. doi:10.1080/17450390902860026.

Hendrix, W. F., K. W. Kelley, C. T. Gaskins, and D. J. Hinrichs. 1978. Porcine Neonatal Survival and Serum Gamma Globulins. J. Anim. Sci. 47:1281–1286. doi:10.2527/jas1978.4761281x.

Heyman, M. 2005. Gut barrier dysfunction in food allergy. Eur. J. Gastroenterol. Hepatol. 17:1279–1285. doi:10.1097/00042737-200512000-00003.

Hidaka, H., T. Eida, T. Takizawa, T. Tokunaga, and Y. Tashiro. 1986. Effects of Fructooligosaccharides on Intestinal Flora and Human Health. Bifidobact. Microflora. 5:37–50. doi:10.12938/bifidus1982.5.1_37.

Holland, R. E. 1990. Some infectious causes of diarrhea in young farm animals. Clin. Microbiol. Rev. 3:345–75. doi:10.1128/CMR.3.4.345.

Hötzel, M. J., G. P. P. de Souza, O. A. D. Costa, and L. C. P. Machado Filho. 2011. Disentangling the effects of weaning stressors on piglets' behaviour and feed intake: Changing the housing and social environment. Appl. Anim. Behav. Sci. 135:44–50. doi:10.1016/J.APPLANIM.2011.09.003.

Houdijk, J. G. M., R. Hartemink, M. W. A. Verstegen, and M. Bosch. 2002. Effects of Dietary Non-Digestible Oligosaccharides on Microbial Characteristics of Ileal Chyme and Faeces in Weaner Pigs. Arch. für Tierernaehrung. 56:297–307. doi:10.1080/00039420214346.

Hu, C. H., K. Xiao, Z. S. Luan, and J. Song. 2013. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs1. J. Anim. Sci. 91:1094–1101. doi:10.2527/jas.2012-5796.

Jenkins, D. J. A., C. W. C. Kendall, and V. Vuksan. 1999. Inulin, Oligofructose and Intestinal Function. J. Nutr. 129:1431S–1433S. doi:10.1093/jn/129.7.1431S.

Jenkins, D. J. A., C. W. C. Kendall, L. S. A. Augustin, M. C. Martini, M. Axelsen, D. Faulkner, E. Vidgen, T. Parker, H. Lau, P. W. Connelly, J. Teitel, W. Singer, A. C. Vandenbroucke, L. A. Leiter, and R. G. Josse. 2002. Effect of wheat bran on glycemic control and risk factors for cardiovascular disease in type 2 diabetes. Diabetes Care. 25:1522–8. doi:10.2337/DIACARE.25.9.1522.

Jensen, A. R., J. Elnif, D. G. Burrin, and P. T. Sangild. 2001. Development of Intestinal Immunoglobulin Absorption and Enzyme Activities in Neonatal Pigs Is Diet Dependent 1. J. Nutr. 131:3259–3265.

Johnson, R. W., and E. von Borell. 1994. Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin. J. Anim. Sci. 72:309–314. doi:10.2527/1994.722309x.

Khoury, D. El, C. Cuda, B. L. Luhovyy, and G. H. Anderson. 2012. Beta Glucan: Health Benefits in Obesity and Metabolic Syndrome. J. Nutr. Metab. 2012. doi:10.1155/2012/851362.

King, D. E., B. M. Egan, R. F. Woolson, A. G. Mainous, Y. Al-Solaiman, and A. Jesri. 2007. Effect of a High-Fiber Diet vs a Fiber-Supplemented Diet on C-Reactive Protein Level. Arch. Intern. Med. 167:502–506. doi:10.1001/archinte.167.5.502.

Klemm, D., B. Heublein, H.-P. Fink, and A. Bohn. 2005. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. Angew. Chemie Int. Ed. 44:3358–3393. doi:10.1002/anie.200460587.

Komai, M., and S. Kimura. 1979. Effects of restricted diet and intestinal flora on the life span of small intestine epithelial cells in mice. J. Nutr. Sci. Vitaminol. (Tokyo). 25:87–94. doi:10.3177/jnsv.25.87.

Krishnamurthy, V. M. R., G. Wei, B. C. Baird, M. Murtaugh, M. B. Chonchol, K. L. Raphael, T. Greene, and S. Beddhu. 2012. High dietary fiber intake is associated with decreased inflammation and all-cause mortality in patients with chronic kidney disease. Kidney Int. 81:300–306. doi:10.1038/KI.2011.355.

Lallès, J.-P., H. M. Tukur, P. Salgado, E. N. C. Mills, Michael R. A. Morgan, L. Quillien, D. Levieux, and R. Toullec. 1999. Immunochemical Studies on Gastric and Intestinal Digestion of Soybean Glycinin and β-Conglycinin in Vivo. 47:2797–2806. doi:10.1021/jf980882+.

Lallès, J.P., G. Boudry, C. Favier, N. Le Floc'h, I. Luron, L. Montagne, I. P. Oswald, S. Pié, C. Piel, and B. Sève. 2004. Gut function and dysfunction in young pigs: physiology. Anim. Res. 53:301–316. doi:10.1051/animres:2004018.

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancock, G. L. Allee, R. D. Goodband, and R. D. Klemm. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J. Anim. Sci. 68:1790–1799. doi:10.2527/1990.6861790x.

Lim, B. O., K. Yamada, M. Nonaka, Y. Kuramoto, P. Hung, and M. Sugano. 1997. Dietary Fibers Modulate Indices of Intestinal Immune Function in Rats. J. Nutr. 127:663–667. doi:10.1093/jn/127.5.663.

Ma, Y., J. A. Griffith, L. Chasan-Taber, B. C. Olendzki, E. Jackson, E. J. Stanek, W. Li, S. L. Pagoto, A. R. Hafner, and I. S. Ockene. 2006. Association between dietary fiber and serum C-reactive protein. Am. J. Clin. Nutr. 83:760–766. doi:10.1093/ajcn/83.4.760.

Madara, J. L., S. Nash, R. Moore, and K. Atisook. 1990. Structure and function of the intestinal epithelial barrier in health and disease. Monogr. Pathol. 31:306–24.

Martin, R., A. Nauta, K. Ben Amor, L. Knippels, J. Knol, and J. Garssen. 2010. Early life: gut microbiota and immune development in infancy. Benef. Microbes. 1:367–382. doi:10.3920/BM2010.0027.

Mavromichalis, I. 2006. Applied Nutrition for Young Pigs. 1st ed. CABi.

Meslin, J.-C., M. Bensaada, F. Popot, and C. Andrieux. 2001. Differential influence of butyrate concentration on proximal and distal colonic mucosa in rats born germ-free and associated with a strain of Clostridium paraputrificum. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 128:379–384. doi:10.1016/S1095-6433(00)00317-2.

Moeser, A. J., C. Vander Klok, K. A. Ryan, J. G. Wooten, D. Little, V. L. Cook, and A. T. Blikslager. 2007a. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am. J. Physiol. Liver Physiol. 292:G173–G181. doi:10.1152/ajpgi.00197.2006.

Moeser, A. J., K. A. Ryan, P. K. Nighot, and A. T. Blikslager. 2007b. Gastrointestinal dysfunction induced by early weaning is attenuated by delayed weaning and mast cell blockade in pigs. Am. J. Physiol. Liver Physiol. 293:G413–G421. doi:10.1152/ajpgi.00304.2006.

Moeser, A. J., C. S. Pohl, and M. Rajput. 2017. Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. Anim. Nutr. 3:313–321. doi:10.1016/J.ANINU.2017.06.003.

Moreno, F. J., and A. Clemente. 2008. 2S Albumin Storage Proteins: What Makes them Food Allergens? Open Biochem. J. 2:16–28. doi:10.2174/1874091X00802010016.

Newberry, R. C., and D. G. M. Wood-Gush. 1988. Development of some behaviour patterns in piglets under semi-natural conditions. Anim. Prod. 46:103–109. doi:10.1017/S0003356100003160.

Newburg, D. S., and W. A. Walker. 2007. Protection of the Neonate by the Innate Immune System of Developing Gut and of Human Milk. Pediatr. Res. 61:2–8. doi:10.1203/01.pdr.0000250274.68571.18.

Nguyen, M., E. Leuridan, T. Zhang, D. De Wit, F. Willems, P. Van Damme, M. Goldman, and S. Goriely. 2010. Acquisition of Adult-Like TLR4 and TLR9 Responses during the First Year of Life. D. Hartl, editor. PLoS One. 5:e10407. doi:10.1371/journal.pone.0010407.

Ogawa, T., N. Bando, H. Tsuji, K. Nishikawa, and K. Kitamura. 1995. α-Subunit of β-Conglycinin, an Allergenic Protein Recognized by IgE Antibodies of Soybean-sensitive Patients with Atopic Dermatitis. Biosci. Biotechnol. Biochem. 59:831–833. doi:10.1271/bbb.59.831.

Oh, I. K., I. Y. Bae, and H. G. Lee. 2014. In vitro starch digestion and cake quality: Impact of the ratio of soluble and insoluble dietary fiber. Int. J. Biol. Macromol. 63:98–103. doi:10.1016/J.IJBIOMAC.2013.10.038.

Pabst, R., M. Geist, H. J. Rothkötter, and F. J. Fritz. 1988. Postnatal development and lymphocyte production of jejunal and ileal Peyer's patches in normal and gnotobiotic pigs. Immunology. 64:539–44.

Pawar, S. N., and K. J. Edgar. 2012. Alginate derivatization: A review of chemistry, properties and applications. Biomaterials. 33:3279–3305. doi:10.1016/J.BIOMATERIALS.2012.01.007.

Perry, J. R., and Ying, W.. 2016. A Review of Physiological Effects of Soluble and Insoluble Dietary Fibers. J. Nutr. Food Sci. 6. doi:10.4172/2155-9600.1000476.

PigCHAMP team. 2019. PigCHAMP Benchmarking USA 2019 summary. Available from: https://www.pigchamp.com/benchmarking/benchmarking-summaries

Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci. 62:131–144. doi:10.1017/S1357729800014417.

Pohl, C. S., J. E. Medland, E. Mackey, L. L. Edwards, K. D. Bagley, M. P. DeWilde, K. J. Williams, and A. J. Moeser. 2017. Early weaning stress induces chronic functional diarrhea, intestinal barrier defects, and increased mast cell activity in a porcine model of early life adversity. Neurogastroenterol. Motil. 29:e13118. doi:10.1111/nmo.13118.

Poonsuk, K., and J. Zimmerman. 2017. Historical and contemporary aspects of maternal immunity in swine. Anim. Heal. Res. Rev. 19:31–45. doi:10.1017/S1466252317000123

Porter, P. 1986. Immune System. In: A. D. Leman, editor. Diseases of Swine. 6th ed. Iowa State University Press. p. 44–57.

Pratt, V. C., K. A. Tappenden, M. I. McBurney, and C. J. Field. 1996. Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition Improves Nonspecific Immunity After Intestinal Resection in Rats. J. Parenter. Enter. Nutr. 20:264–271. doi:10.1177/0148607196020004264.

Qiao, S., D. Li, J. Jiang, H. Zhou, J. Li, and P. A. Thacker. 2003. Effects of Moist Extruded Full-fat Soybeans on Gut Morphology and Mucosal Cell Turnover Time of Weanling Pigs. Asian-Australasian J. Anim. Sci. 16:63–69. doi:10.5713/ajas.2003.63.

Romagnani, S. 1992. Type 1 T helper and type 2 T helper cells: Functions, regulation and role in protection and disease. Int. J. Clin. Lab. Res. 21:152–158. doi:10.1007/BF02591635.

Rossi, F., E. Cox, B. Goddeeris, D. Portetelle, J. Wavreille, and A. Thewis. 2001. Inulin incorporation in the weaned pig diet: intestinal coliform interaction and effect on specific systemic immunity. In: J. E. Lindberg and B. Ogle, editors. Digestive Physiology of Pigs : Proceedings of the 8th Symposium. CABI Pub, New York. p. 299–301.

Satchithanandam, S., M. Vargofcak-Apker, R. J. Calvert, A. R. Leeds, and M. M. Cassidy. 1990. Alteration of Gastrointestinal Mucin by Fiber Feeding in Rats. J. Nutr. 120:1179–1184. doi:10.1093/jn/120.10.1179.

Segain, J.-P. 2000. Butyrate inhibits inflammatory responses through NFkappa B inhibition: implications for Crohn's disease. Gut. 47:397–403. doi:10.1136/gut.47.3.397.

Sève, B., P. J. Reeds, M. F. Fuller, A. Cadenhead, and S. M. Hay. 1986. Protein synthesis and retention in some tissues of the young pig as influenced by dietary protein intake after early-weaning. Possible connection to the energy metabolism. Reprod. Nutr. Développement. 26:849–861. doi:10.1051/rnd:19860509.

Shields, R. G., K. E. Ekstrom, and D. C. Mahan. 1980. Effect of Weaning Age and Feeding Method on Digestive Enzyme Development in Swine from Birth to Ten Weeks. J. Anim. Sci. 50:257–265. doi:10.2527/jas1980.502257x.

Skrzypek, T., J. L. Valverde Piedra, H. Skrzypek, W. Kazimierczak, S. E. Szymanczyk, and R. Zabielski. 2010. Changes in pig small intestinal absorptive area during the first 14 days of life ☆. Livest. Sci. 133:53–56. doi:10.1016/j.livsci.2010.06.023.

Stokes, C., and J. F. Bourne. 1989. Mucosal Immunity. In: R. E. W. Halliwell and N. T. Gorman, editors. Veterinary Clinical immunology. p. 164–192.

Sun, P., D. Li, B. Dong, S. Qiao, and X. Ma. 2008. Effects of soybean glycinin on performance and immune function in early weaned pigs. Arch. Anim. Nutr. 62:313–321. doi:10.1080/17450390802066419.

Sun, L., C. He, L. Nair, J. Yeung, and C. E. Egwuagu. 2015. Interleukin 12 (IL-12) family cytokines: Role in immune pathogenesis and treatment of CNS autoimmune disease. Cytokine. 75:249–55. doi:10.1016/j.cyto.2015.01.030.

Superchi, P., R. Saleri, P. Borghetti, G. Ferrarini, V. Cavalli, M. Sereni, S. Zavattini, and A. Sabbioni. 2017. Effects of a dietary crude fibre concentrate on growth in weaned piglets. Animal. 11:1905–1912. doi:10.1017/S175173111700057X.

Svendsen, L. S., B. R. Weström, J. Svendsen, A.C. Olsson, and B. W. Karlsson. 1990. Intestinal macromolecular transmission in underprivileged and unaffected newborn pigs: implication for survival of underprivileged pigs. Res. Vet. Sci. 48:184–189. doi:10.1016/S0034-5288(18)30988-3.

Tanaka, R., H. Takayama, M. Morotomi, T. Kuroshima, S. Ueyama, K. Matsumoto, A. Kuroda, and M. Mutai. 2014. Effects of Administration of TOS and Bifidobacterium breve 4006 on the Human Fecal Flora. Bifidobact. Microflora. 2:17–24. doi:10.12938/bifidus1982.2.1_17.

Tarvid, I., P. D. Cranwell, L. Ma, and R. Vavala. 1994. The early postnatal development of protein digestion in pigs. II. Small intestinal enzymes. In: VIth International Symposium on Digestive Physiology in Pigs. VI. p. 181–184.

Theuwissen, E., and R. P. Mensink. 2008. Water-soluble dietary fibers and cardiovascular disease. Physiol. Behav. 94:285–292. doi:10.1016/J.PHYSBEH.2008.01.001.

Trowell, H. 1972. Ischemic heart disease and dietary fiber. Am. J. Clin. Nutr. 25:926–932. doi:10.1093/ajcn/25.9.926.

Trowell, H. 1973. Dietary fibre, ischaemic heart disease and diabetes mellitus. Proc. Nutr. Soc. 32:151–157. doi:10.1079/PNS19730033.

van Beers-Schreurs, H. M. G., M. J. A. Nabuurs, L. Vellenga, H. J. K. der Valk, T. Wensing, and H. J. Breukink. 1998. Weaning and the Weanling Diet Influence the Villous Height and Crypt Depth in the Small Intestine of Pigs and Alter the Concentrations of Short-Chain Fatty Acids in the Large Intestine and Blood. J. Nutr. 128:947–953. doi:10.1093/jn/128.6.947.

Vodovar, N., J. Flanzy, and A. C. François. 1964. INTESTIN GRÊLE DU PORC ÉTUDE DE LA JONCTION DU CANAL CHOLÉDOQUE ET DU CANAL PANCRÉATIQUE A CELUI-CI.

Vos, A. P., L. M'Rabet, B. Stahl, G. Boehm, and J. Garssen. 2007. Immune-Modulatory Effects and Potential Working Mechanisms of Orally Applied Nondigestible Carbohydrates. Crit. Rev. Immunol. 27:97–140. doi:10.1615/CritRevImmunol.v27.i2.10.

Wang, X., and G. R. Gibson. 1993. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J. Appl. Bacteriol. 75:373–380. doi:10.1111/j.1365-2672.1993.tb02790.x.

Wang, X., F. Geng, J. Wu, Y. Kou, S. Xu, Z. Sun, S. Feng, L. Ma, and Y. Luo. 2014. Effects of β -conglycinin on growth performance, immunoglobulins and intestinal mucosal morphology in piglets. Arch. Anim. Nutr. 68:186–195. doi:10.1080/1745039X.2014.919733.

Weickert, M. O., and A. F. H. Pfeiffer. 2008. Metabolic Effects of Dietary Fiber Consumption and Prevention of Diabetes. J. Nutr. 138:439–442. doi:10.1093/jn/138.3.439.

Weström, B. R., B. G. Ohlsson, J. Svendsen, C. Tagesson, and B. W. Karlsson. 1985. Intestinal Transmission of Macromolecules (BSA and FITC-Dextran) in the Neonatal Pig: Enhancing Effect of Colostrum, Proteins and Proteinase Inhibitors. Neonatology. 47:359–366. doi:10.1159/000242140.

Wijtten, P. J. A., J. Van Der Meulen, and M. W. A. Verstegen. 2011. Intestinal barrier function and absorption in pigs after weaning: A review. Br. J. Nutr. 105:967–981. doi:10.1017/S0007114510005660.

Wiyaporn, M., B. Thongsong, and S. Kalandakanond-Thongsong. 2013. Growth and small intestine histomorphology of low and normal birth weight piglets during the early suckling period. Livest. Sci. 158:215–222. doi:10.1016/j.livsci.2013.10.016.

Wu, H.-J., and E. Wu. 2012. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes. 3:4–14. doi:10.4161/gmic.19320.

Wu, Y., W. Li, W. Cui, N. A. M. Eskin, and H. D. Goff. 2012. A molecular modeling approach to understand conformation–functionality relationships of galactomannans with different mannose/galactose ratios. Food Hydrocoll. 26:359–364. doi:10.1016/J.FOODHYD.2011.02.029.

Wu, J. J., C. M. Cao, D. D. Ren, Y. Zhang, Y. N. Kou, L. Y. Ma, S. Ben Feng, Y. Li, and X. C. Wang. 2016. Effects of soybean antigen proteins on intestinal permeability, 5-hydroxytryptamine levels and secretory IgA distribution in the intestine of weaned piglets. Ital. J. Anim. Sci. 15:174–180. doi:10.1080/1828051X.2016.1148559.

Xu, R.-J., Q. C. Doan, and G. O. Regester. 2004. Role of transforming growth factor-β in breast milk for initiation of IgA production in newborn infants. Early Hum. Dev. 77:67–75. doi:10.1016/J.EARLHUMDEV.2004.01.005.

Yamada, K., Y. Tokunaga, A. Ikeda, K. Ohkura, S. Mamiya, S. Kaku, M. Sugano, and H. Tachibana. 1999. Dietary effect of guar gum and its partially hydrolyzed product on the lipid metabolism and immune function of sprague-dawley rats. Biosci. Biotechnol. Biochem. 63:2163–2167. doi:10.1271/bbb.63.2163.

Yun, C.-H., A. Estrada, A. van Kessel, A. A. Gajadhar, M. J. Redmond, and B. Laarveld. 1997. β-(1→3, 1→4) Oat glucan enhances resistance to Eimeria vermiformis infection in immunosuppressed mice. Int. J. Parasitol. 27:329–337. doi:10.1016/S0020-7519(96)00178-6.

Zhang, J., G. Wu, R. S. Chapkin, and J. R. Lupton. 1998. Energy Metabolism of Rat Colonocytes Changes during the Tumorigenic Process and Is Dependent on Diet and Carcinogen. J. Nutr. 128:1262–1269. doi:10.1093/jn/128.8.1262.

Zhao, Y., G. Qin, R. Han, J. Wang, X. Zhang, and D. Liu. 2014. β-conglycinin reduces the tight junction occludin and ZO-1 expression in IPEC-J2. Int. J. Mol. Sci. 15:1915–1926. doi:10.3390/ijms15021915.

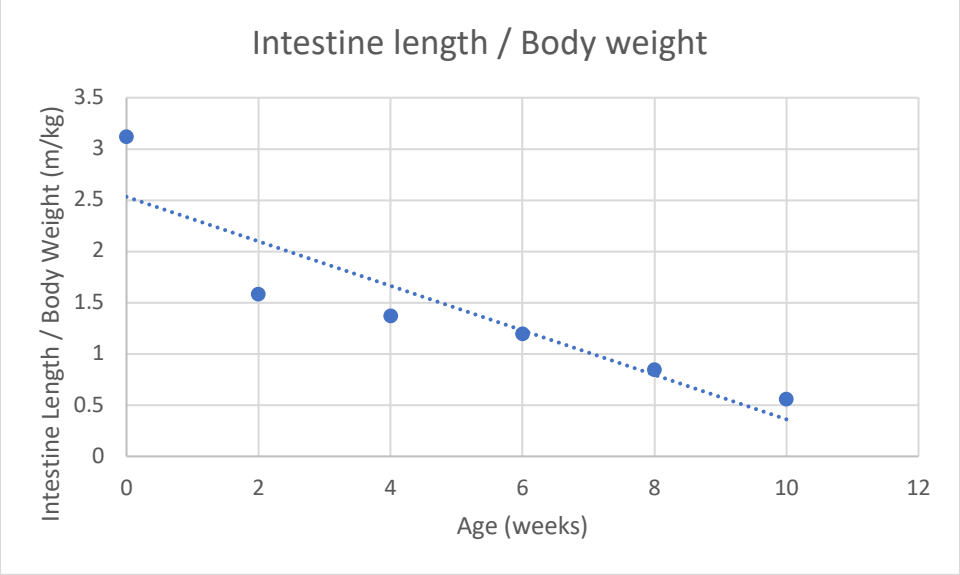


Figure 1.1 Ratio of intestine length/bodyweight in pigs from birth to ten weeks of age (Adapted from Shields et al., 1980)

CHAPTER 2. EFFECTS OF FEEDING SOLUBLE FIBER (DEXTRIN) TO PIGS PRE- AND POST-WEANING ON THE INTESTINAL MICROBIOME, VOLATILE FATTY ACID (VFA) PRODUCTION, INTESTINAL MORPHOLOGY, AND GENE EXPRESSION

2.1 Abstract

Forty barrows were used in a 35d experiment to evaluate the effects of supplemental soluble fiber (dextrin) pre- and post-weaning on growth performance, intestinal microbiome, volatile fatty acid (VFA) production, intestinal morphology, and gene expression. Pigs were blocked by litter and BW, and randomly allotted to treatments in a 2x2 factorial design with or without fiber pre-weaning and with or without fiber post-weaning. Dextrin was administered orally through a syringe, after being suspended in chocolate milk from 14d prior to weaning through 3d post-weaning, after which it was included in the diet at 1%. At weaning, pigs were group housed by treatment and allowed ad libitum access to a common starter diet. On d4 post-weaning, pigs were moved to individual pens and fed diets with or without 1% fiber. Weights and feed intake were recorded 14 and 3d prior to weaning, and on d0, 4, 11, and 21 post-weaning. On d0 and d21 post-weaning, pigs were euthanized for collection of tissues and intestinal contents. Ileal, cecal, and colon contents were taken for microbiome analysis, distal large intestine contents were collected for VFA analysis, ileal cross sections were collected for histology, and ileal and cecal mucosal scrapings were collected for intestinal gene expression. Data were analyzed using the GLM procedure of SAS with pig as the experimental unit for growth performance, VFA production, intestinal morphology, and gene expression. Microbiome data were analyzed using Metastats, to find statistical significance between treatments, and then run through R, using the false discovery rate method, to find a multiple test corrections q-value. Growth performance in general was not affected ($P > 0.10$) by treatment with the exception of

d11-21 feed efficiency was improved ($P = 0.018$) for pigs receiving supplemental fiber prior to weaning. Pigs that received fiber at any point had increased short chain fatty acid (SCFA) producing bacteria ($q < 0.05$) compared to pigs never receiving fiber. Pigs never receiving fiber had increased bacteria associated with intestinal inflammation ($q < 0.05$) compared to all other treatment groups. A trend for an interaction ($P = 0.054$) of pre- and post-weaning fiber supplementation was observed for total volatile fatty acid concentration in large intestine contents. An interaction ($P = 0.007$) of pre- and post-weaning treatments was observed on butyrate, with pigs fed fiber only during pre-weaning having the greatest butyrate concentrations. Pigs fed fiber pre-weaning had decreased isobutyrate concentrations ($P = 0.050$) and percentages ($P =0.040$) and a trend for decreased isovalerate as a concentration ($P = 0.058$) and percent of total VFAs ($P = 0.051$). Pigs fed fiber post-weaning had increased acetate ($P = 0.047$). An interaction for butyrate percentages was observed with pigs receiving supplemental fiber only prior to weaning having the highest percent of butyrate ($P = 0.029$). An interaction for valerate concentrations ($P = 0.045$) occurred with pigs receiving fiber only prior to weaning having the highest amount of valerate. Valerate as a proportion of total VFAs ($P = 0.038$) was decreased in pigs receiving supplemental fiber post-weaning. Pigs fed fiber prior to weaning tended to have decreased crypt depths ($P = 0.097$) compared to pigs that did not receive fiber prior to weaning. In the ileum there was an interaction ($P = 0.002$) for GLP-2 expression, with pigs receiving supplemental fiber solely before or after weaning having the greatest expression. Occludin expression in the ileum tended to increase with fiber supplementation prior to weaning ($P = 0.086$) but then tended to decrease with fiber supplementation post-weaning ($P = 0.053$) In the cecum, there was an interaction ($P = 0.049$) of pre- and post-weaning fiber supplementation on GLP-2 expression. Pigs fed supplemental fiber at any point had increased GLP-2 expression, but

pigs that had fiber only after weaning had the greatest GLP-2 expression. Cecal HSP-70 expression also increased with fiber supplementation in pigs fed fiber post-weaning ($P = 0.012$). Soluble fiber supplementation caused alterations in the intestinal microbiome, VFA concentrations, the intestinal morphology, and in the expression of different intestinal genes.

2.2 Introduction

Weaning is one of the more stressful times in the life of a pig and is associated with many gastrointestinal alterations including reduced villus heights, increased crypt depths, changes to the intestinal microbiome, and poor regulation of an underdeveloped immune system (Hampson, 1986; Pluske et al., 1997; Heo et al., 2013). These alterations often cause diarrhea, decreased absorptive capacity, increased intestinal inflammation, and decreased growth performance following weaning (Pluske et al., 1996; Wijtten et al., 2011; Campbell et al., 2013). These effects usually occur due to sudden environmental, social, and dietary changes around the time of weaning (Lalles et al., 2007; Hötzel et al., 2011). Some of these changes are attributed to stress (Moeser et al., 2007) while some are attributed to decreased feed intake (Pluske et al., 1997). However, a study done by Kelly et al. (1991) reported reduced villus heights and increased crypt depths in pigs after weaning when fed through gastric intubation, indicating that the reduced absorptive capacity was not due to decreased feed intake, but rather to other stressors that occur around weaning. An increased incidence of diarrhea following weaning is usually credited to the proliferation of *Escherichia coli* which may be exacerbated by decreased barrier function causing an imbalanced microbiome and pathogenic infection (Fairbrother et al., 2005; Chen et al., 2013). Antibiotics are added to the feed to treat or prevent infections in the pig and to combat the negative effects of weaning. However, antibiotic use in livestock production is coming under increased public scrutiny due to concerns over antibiotic resistance, for both humans and pigs,

and there is a serious push by the industry, fueled by pressure from the public, to reduce antibiotic usage (Van Boeckel et al., 2015). A multitude of new strategies have been researched to replace the use of antibiotics in feed to decrease intestinal permeability, reduce diarrhea, increase absorptive capacity, and increase growth performance.

An increase in intestinal permeability can be due to a reduced concentration of tight junction proteins, such as occludin, in the intestinal mucosa (Oswald, 2006). When tight junction protein expression is decreased, pathogenic substances can pass through the intestinal wall paracellularly and activate the immune system (Turner, 2006), which can cause intestinal inflammation (Heyman, 2005; Campbell et al., 2013). Intestinal permeability and integrity can be altered by pro-inflammatory cytokines (McKay and Baird, 1999). Heat shock proteins (HSP) function in the body is to cause reparation in tissue following some form of damage and HSPs increase in pigs during the first 24 h post-weaning (Lallès et al., 2004; Bianchi, 2007). Glucagon-Like Peptide 2 (GLP-2) has been reported to aid in the reduction of intestinal permeability and inflammation, which are prevalent symptoms following weaning, and increase crypt cell proliferation while suppressing cell apoptosis in intestinal epithelial cells (Burrin et al., 2003). Volatile fatty acids (VFAs) are produced by microbes and an alteration in VFA concentrations typically indicates an alteration in microbe concentrations or organisms (Franklin et a., 2002). Microbiota in the gut have proven to be an important aspect involved in the overall health and development of the pig (Fouhse et al., 2016).

Fiber has many beneficial effects on the health of the pig. Bauer et al. (2006) reported that fiber alters the gut microbiome and acts as a prebiotic source, boosting beneficial bacterial while inhibiting the growth of some pathogenic bacteria. Wang and Gibson (1993) reported a similar conclusion after incubating fructo-oligosaccharides and inulin with human feces which

subsequently decreased the pH of the feces and decreased *Clostridium perfringens* and *E. coli* concentrations. Fiber has also been reported to reduce gut inflammation and reduce intestinal permeability (King et al., 2007; Grooms et al., 2013), which could limit the number of harmful bacteria that are able to cross the intestinal barrier around the time of weaning. Dextrin, the soluble fiber studied in this experiment, consists of multiple glucose monomers linked by α-1,4 linkages (Takata et al., 2005). The purpose of this study was to include dextrin in the diet of pigs pre- and post-weaning to test the ability of supplemental soluble fiber to alleviate negative health symptoms that occur shortly after weaning.

2.3 Materials and Methods

2.3.1 Animals

All animal procedures were approved by the Purdue Animal Care and Use Committee (PACUC #1303000841). This study involved a total of 40 barrows that began the study at 9.2 ± 1 d of age. Three days after trial initiation, one control pig was euthanized due to insufficient nursing. Pigs were a Landrace, Yorkshire, and Chester White composite that were genetically selected to have an increased susceptibility to soybean proteins over 10+ generations (Cabrix et al., 2011). The study lasted for 5 weeks from 14 d prior to weaning until 21 d post-weaning. Before pigs were weaned, pigs were blocked by BW, litter, and lactation treatment, and randomly allotted within block to complete a 2x2 factorial experiment with or without supplemental soluble fiber pre-weaning and with or without supplemental soluble fiber post-weaning. Pigs were weighed on d -14, -3, 0, 4, 11, and 21 relative to weaning (d 0). From d -14 to 0 pigs were housed with the sow in their respective farrowing crate. The morning of d 0 pigs were weaned and moved to group housed pens (1.37 m x 1.52 m) by treatment. On the morning

of d 4 pigs were moved to individual pens (0.41 m x 0.86 m) where they remained for the remainder of the study.

2.3.2 Treatments

Treatments were:

1) Pre-weaning: No supplemental soluble fiber, Post-weaning: No supplemental soluble fiber
2) Pre-weaning: Supplemental soluble fiber, Post-weaning: No supplemental soluble fiber
3) Pre-weaning: No supplemental soluble fiber, Post-weaning: Supplemental soluble fiber
4) Pre-weaning: Supplemental soluble fiber, Post-weaning: Supplemental soluble fiber

For pigs receiving supplemental soluble fiber, it was diluted in chocolate milk warmed to 39°C and administered orally from 14 days prior to weaning through 3 d post-weaning. From d4 to d21 post-weaning, soluble fiber was supplied in the feed. Fiber was supplied to pigs at a rate of 1 g/d dissolved in 5 mL of chocolate milk on days -14 to -8, 2 g/d dissolved in 7.5 mL of chocolate milk on d -7 to -1, and 3 g/d dissolved in 10 mL of chocolate milk on d -1 through d 3 relative to weaning (d 0). Starting on d 4 supplemental fiber was mixed into the feed at 1% of the diet. Pigs not receiving supplemental fiber during the oral dosing period received chocolate milk with no supplemental fiber added.

After weaning on d 0, all pigs were fed a common Phase 1 nursery diet with no supplemental fiber added until d 4 post-weaning when the Phase 2 diet started as pigs were moved to individual housing. During this time (d 0-4), pigs on fiber treatments were given oral doses. All diets were formulated to meet or exceed the minimal nutrient requirements (Table 2.1)

according to the NRC (2012). Diets were analyzed at Purdue University for energy, DM, ash, nitrogen, phosphorous, NDF, and ADF (Table 2.2). The Phase 1 diet had no additional supplemental fiber and was a corn-soy based diet with lactose and animal protein sources (Table 2.1).

On day 4 post-weaning, all pigs were moved to individual housing, and switched from Phase 1 to Phase 2 feed. The Phase 2 diet was a corn-soy based diet with animal protein sources. A basal diet was made without supplemental fiber, and pigs receiving fiber received the basal diet blended with supplemental fiber at a level of 1% of the diet, whereas pigs not receiving supplemental fiber had an extra 1% corn blended into the diet (Table 2.1).

Phase 3 nursery diets began on d 11 and were fed until the conclusion of the study (d 21). Phase 3 was similar to Phase 2 in that supplemental fiber was added to the basal diet at 1% of the diet for pigs receiving supplemental fiber, whereas an extra 1% of corn was added to the diet of pigs not receiving supplemental fiber (Table 2.1).

2.3.3 Animal Growth and Performance

Pigs were weighed at the beginning of the study and on d -3, 0, 4, 11, and 21. All feeder weights were collected on the same day that body weights were collected to calculate feed disappearance. From the morning of day 0 to the morning of day 4, while they were group housed with 7 or 8 pigs/pen, all feeders were initially weighed while empty, feed was weighed and added, then the weight of the feeder and feed was weighed on day 4 to record feed disappearance for the entire pen.

Beginning on day 4, once pigs were placed into individual pens, each pig was assigned their own feed tub containing a pre-weighed amount of feed and feed was added to each feeder, from their corresponding tub, whenever it began to run low. When body weights were collected,

the remaining feed in the tub and feeder was also weighed to record feed disappearance since the previous weigh day. Each pig's weight and feed disappearance were recorded to measure body weight, and calculate average daily gain, average daily feed intake, and gain:feed, for each dietary phase and for the overall growth performance of the study.

2.3.4 Euthanasia and Sample Collection

All pigs were harvested on site at the Purdue University Animal Sciences Research and Education Center and were euthanized using carbon dioxide stunning followed by exsanguination. On the morning of d 0 and 21, 8 and 31 pigs, respectively, were euthanized and tissues were collected from the ileum and cecum while contents were taken from the ileum, cecum, and colon. Figure 2.1 shows the location along the digestive tract where each sample was collected. A 2-inch cross section was taken from the ileum, rinsed with phosphate-buffered saline (PBS), and then placed in 30 mL of 10% neutral buffered formalin for subsequent histological analyses. Ileal and cecal tissue were removed, the lumen was lightly rinsed with PBS and then mucosa was scraped and flash frozen in 1 mL of Invitrogen™ TRIzol® reagent (ThermoFisher Scientific; Waltham, MA, USA) for subsequent isolation of mRNA, creation of cDNA, and analysis of gene expression using quantitative PCR. Digesta was taken from the distal end of the ileum, the cecum, and the large intestine, where it was placed on ice and then frozen at -20°C for subsequent microbiome analysis. After the pigs were euthanized on d 21, fecal samples were taken, which were placed in a sterile bag, then placed on ice until it could be moved to a -20°C freezer for subsequent VFA analysis through gas chromatography.

2.3.5 Histology

Tissue sections that were placed in formalin were then sent to the Purdue University Histology Lab in the College of Veterinary Medicine. The Histology Lab embedded the tissue in

paraffin wax, used a microtome to slice cross sections, and then fixed the tissue to slides prior to staining with hematoxylin and eosin. Digital pictures of the slides were taken with MotiConnect software (version 1.5.9.1 ; Motic China Group Co. Ltd.; Xiamen, China). The digitized slides were then analyzed using ImageJ 1.51k measurement software (LOCI, University of Wisconsin). Six villi and six crypts were measured for each pig. The villi were measured from the tip of the villus down to the base of the villus. Crypts were measured from the base of the villus down to the bottom of the crypt region. An example of this is shown in Figure 2.2.

2.3.6 Microbiome Analysis

All microbiome analysis was performed by at Purdue University. DNA extraction was done using the DNeasy PowerLyzer PowerSoil Kit (Qiagen) following the manufacturer's protocol with two changes. First, during step 4 (lysis), bead tubes were run at 30Hz (1/s) for 2 minutes, and then tube racks were flipped over to ensure even lysis from outer and inner tubes. The second change occurred during step 17 (DNA elution), where instead of 100 µL of the given solution C6, DNA was eluted with 50 µL of nuclease free water. Extracted DNA was used for the construction of a 16S rRNA gene library following a standardized protocol (Kozich et al., 2013). The PCR and sequencing quality were assessed by preparing 16S rRNA gene libraries for a known positive control mock community (20 Strain Even Mix Genomic Material; ATCC® MSA-1002TM) and water as a negative control (Kozich et al. 2013). Briefly, Illumina indexed reads were created using PCR amplification of the V4 region of bacterial 16S rRNA gene. Amplification success was determined through gel electrophoresis as a quality check. No bands were observed in the negative control samples using water as the DNA template. Amplified DNA was normalized using a SequalPrep Normalization Plate (Invitrogen), and pooled into a single library. Library concentration was determined using the KAPA Library Quantification Kit

(Roche) and library average fragment length was determined using the Bioanalyzer (Agilent) with a high sensitivity kit. Following the confirmation of proper DNA concentration, the pooled samples, mock community, and water, were sequenced (Illumina, MiSeq v2 kit, 500 cycle). Sequences were demultiplexed according to oligonucleotide bar code sequence with Illumina software. All equipment for extraction, amplification, and sequencing are located within Purdue University.

Raw reads were analyzed using Mothur (v 1.39.3). The general pipeline for Mothur is as follows: make contigs from raw reads, align contigs to reference sequences (Quast et al. 2012; SILVA database release 132), screen and filter sequences to remove low quality reads (ambiguous bases allowed = 0, maximum read length = 275, homopolymers allowed = 8), group sequences based on sequence similarity, classify sequences with reference to known taxonomic classifications (Cole et al. 2013; RDP training set 16), cluster sequences, and run diversity metrics.

2.3.7 Intestinal Tissue Gene Expression

The mucosa frozen in TRIzol® was removed from a -80°C freezer to isolate RNA. The RNA from the ileal and cecal mucosal scrapings was isolated using a commercially available kit (PureLink™ RNA Mini Kit; Ambion by Life Technologies, Carlsbad, CA, USA). The RNA from each sample was quantified using a NanoDrop spectrophotometer at 260 nm absorbance (ND-100, NanoDrop Technologies, Rockland, DE). The purity of the RNA was determined based on the absorbance ratio of 260 to 280 nm, which had to be 1.9 or higher to be considered sufficient (Wilfinger, 1997). Once RNA was quantified, it was diluted to have 1 µg of RNA to 4.75 µL of nuclease free water. The RNA was then reverse transcribed into cDNA by combining 2.0 µL of RT-buffer, 0.25 µL of Promega RNasin® (Promega Corporation; Madison WI, USA),

0.5 µL of Invitrogen™ M-MLV reverse transcriptase, 1.0 µL of oligo-DT (0.1 mg/mL), 1.0 µL of Bovine Serum Albumin (1 mg / mL), and 0.5 µL of dNTP (ThermoFisher Scientific; Waltham, MA, USA) with 1.0 µg of RNA. RT-qPCR was carried out with the cDNA using 5 µL of sample mixed with 15 µL of 2X SYBR Green supermix (iQ™ SYBR® green supermix, Bio-Rad, Hercules, CA) blended with forward and reverse primers for each target gene to be amplified (Table 2.3). The RT-qPCR machine began with a 95ºC denaturation step followed by 40 cycles of 95ºC for 15 seconds, 60ºC for 60 seconds, and 72ºC for 30 seconds for the genes GAPDH, Occludin, HSP70, and GLP-2 (StepOne Plus, Applied Biosystems™, Carlsbad, CA, USA). TNF-α was analyzed with 40 cycles of 95ºC for 15 seconds, 50ºC for 60 seconds, and 72ºC for 30 seconds and GAPDH was included on these plates as well due to its role as a housekeeper gene. After 40 cycles of PCR, a melt curve was generated to check primer specificity. To minimize error, each sample was amplified in duplicate on the same plate for all primers, and a pooled composite, non-template sample was used as a control and included on each plate to account for any possible plate-to-plate variability. GAPDH was used as a housekeeper gene to normalize the sample using the ΔΔCT method. Primers used for occludin, HSP70, GAPDH, and GLP-2 are shown in Table 2.3.

2.3.8 Volatile Fatty Acid (VFA) Analysis

Fecal samples were removed from the freezer and thawed in a refrigerator. Thawed feces were diluted with a recorded amount of deionized (DI) water until they resembled more of a liquid, and then mixed with 2 mL of 25% metaphosphoric acid and placed into tubes. Each sample was divided into 2 tubes so that each sample could be run in duplicate. Samples were then vortexed for 10 seconds, placed into a centrifuge and spun at 15,120 x g for 10 minutes. From this sample the supernatant was collected. The samples were centrifuged again at 15,120 x

g for 10 minutes and the supernatant was collected and poured into a 12-cc syringe, which connected to a paper filter with a pore size of 0.45 μm. The supernatant was passed through the filter and that sample was collected. A stock solution with 87.4 μM/L acetic, 26.76 μM/L propionic, 7.607 μM/L butyric, 8.789 μM/L isobutyric, 1.864 μM/L valeric, and 1.835 μM/L isovaleric acid was mixed as a standard to normalize the samples. The Gas Chromatograph (3900 CP-8400, Varian Medical Systems™, Palo Alto, CA, USA) was loaded with a blank containing a 1:5 dilution of 25% metaphosphoric acid and a standard containing a 1:5 dilution of the standard stock. All samples were then loaded into the machine and run through a fused silica capillary column measuring 0.25mm x 0.25μm x 30 m. The sample analysis was run using Galaxie analysis software (Galaxie, Varian Medical Systems™, Palo Alto, CA, USA).

2.3.9 Statistical Analysis

For pig growth performance, intestinal morphology, and intestinal gene expression pre-weaning, pigs were euthanized on day 0 (n=8). All data were analyzed with two treatment groups, with and without supplemental fiber. For pigs euthanized on day 21, the pig was used as the experimental unit (n=31) for growth performance, gene expression, histology and VFA concentrations. Data were analyzed using PROC GLM in SAS 9.4 (SAS Inst. Inc., Cary, NC). All data were analyzed as a 2x2 factorial arrangement with or without supplemental fiber pre-weaning and with or without supplemental fiber post-weaning. Values were considered significant at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$. All data were analyzed for outliers over the entire study group. Any data above or below 2.5 standard deviations from the average was removed and not included in statistical analysis. Four additional pigs, one per treatment, were removed from the final data set for analysis due to poor health. For microbiome data, Metastats (Schloss et al. 2009) was used to determine statistical significance between treatment groups and

a multiple test correction q-value, calculated using the false discovery rate method, was done using R (R Core Team 2013).

2.4 Results

2.4.1 Animal Growth and Performance

During the nursing period there was no effect of fiber supplementation on ADG or BW at any point during lactation ($P > 0.510$; Table 2.4). There were not any differences in ADG while pigs were eating solid feed between d0 and d4 ($P \geq 0.261$; Table 2.5). Since the pigs were group housed d 0-4 post-weaning, ADFI was based on the pen, not the individual pig. Since this data has n=1 there are no p-values, but it is shown in Table 2.5 for reference. From d4 to d11, while pigs were individually housed, there were no differences among treatment groups for ADG, ADFI, G:F, d 4 BW, or d 11 BW for this period ($P \geq 0.227$; Table 2.5). From d11 to d21 there were no differences in ADG, ADFI, or d21 BW ($P \geq 0.253$; Table 2.5), however there was a pre-weaning fiber effect on G:F with pigs receiving supplemental fiber prior to weaning having an increased G:F ($P = 0.018$; Table 2.5). During the overall post-weaning period, ADG was assessed from d0 to 21 and d4 to 21, while ADFI, and G:F were assessed from d4 to d21 since no individual feed intake data could be collected from d 0 to4 while pigs were group housed. Average daily gain from d0 to 21 and d4 to 21, and ADFI, and G:F from day 4 to day 21 were not different among treatment groups ($P \geq 0.182$; Table 2.5).

2.4.2 Histology

Pigs euthanized at weaning on d0 had no differences between treatment groups for villus height, crypt depth, or villus height:crypt depth ($P \geq 0.355$; Table 2.4). However. differences in intestinal histology were recorded in pigs euthanized at the end of the study on d 21. Providing

supplemental fiber to pigs prior to weaning tended to decrease crypt depth ($P = 0.097$), while villus height ($P = 0.781$; Table 2.6) and villus height:crypt depth ratio ($P = 0.117$; Table 2.6) both remained unchanged. The addition of supplemental fiber after weaning resulted in no differences in villus height, crypt depth, or villus height:crypt depth ($P \geq 0.198$; Table 2.7). There were no diet x stage of supplementation interactions on villus height, crypt depth, or villus height:crypt depth ($P \geq 0.265$; Table 2.8).

2.4.3 Microbiome Analysis

The top 16 genera based on relative abundance in the the ileum, cecum, and colon, were the same between pre- and post-weaning pigs (Figure 2.3). The top 16 included lactic acid bacteria (LAB) such as *Lactobacillus*, *Megasphaera*, *Streptococcus* and *Bifidobacteria* as well as bacteria that may induce inflammation and disease such as *Helicobacter* and *Campylobacter* (Figure 2.3).

No differences were observed between the pigs given soluble fiber or not and euthanized prior to weaning for any sample type (Figure 2.3). Pigs that received supplemental fiber had increased SCFA producing bacteria ($q < 0.05$) compared to pigs that never received supplemental fiber in the cecum and colon for both mucosal and digesta samples (Table 2.9). The SCFA producing bacteria that increased are genera such as *Butyrivibrio*, *Butyricimonas*, *Acetivibrio*, and *Turicibacter* (Azman et al., 2015; Ulger Toprak et al., 2015; Zhong et al., 2015). Pigs that never received supplemental fiber had increased *Desulfovibrionaceae* ($q < 0.05$), a family with members implicated in increased inflammation (Figliuolo et al., 2017), in both cecum and colon mucosal samples.

2.4.4 Gene Expression

Pigs euthanized on d0 had no changes in GLP-2, HSP-70, or occludin expression in the cecum ($P \geq 0.367$; Table 2.4), and no changes in GLP-2 or occludin were observed in the ileum ($P \geq 0.402$; Table 2.4). For pigs harvested on d 21, adding supplemental fiber to the diet caused a diet by stage of supplementation interaction for ileal GLP-2 gene expression. This resulted from pigs that received supplemental fiber either pre- or post-weaning, but not both times, having greater GLP-2 gene expression than pigs that never received fiber ($P = 0.002$; Figure 2.4). Fiber supplementation also caused a trend for a pre-weaning effect ($P = 0.086$) and a trend for a post weaning effect ($P = 0.053$) on occludin gene expression in pigs euthanized on d21, with pigs being fed supplemental fiber prior to weaning having increased occludin expression, but pigs being fed supplemental fiber post-weaning having decreased occludin expression (Figure 2.5). There was a diet x stage of supplementation interaction for cecal GLP-2 expression ($P = 0.049$; Figure 2.6). Pigs not fed supplemental fiber pre-weaning, but receiving supplemental fiber post-weaning had a greater increase in cecal GLP-2 expression than pigs given supplemental fiber both pre- and post-weaning and compared to pigs only receiving supplemental fiber pre-weaning. There was a post-weaning main effect on cecal HSP-70 gene expression with pigs receiving supplemental fiber post-weaning having higher HSP-70 gene expression ($P = 0.012$; Figure 2.7) Occludin expression in the cecum was not affected by fiber supplementation ($P \geq 0.121$; Figure 2.8).

2.4.5 Volatile Fatty Acid (VFA) Analysis

For pigs euthanized on d21, adding fiber to the diet caused a diet x stage of supplementation interaction for the total amount of VFAs ($P = 0.054$; Table 2.10). While pigs receiving supplemental fiber at any point had greater concentrations of total VFAs compared to

pigs that never received supplemental fiber, the interaction is due to pigs receiving fiber solely prior to weaning having increased total VFA concentrations compared to pigs that received supplemental fiber both pre- and post-weaning. Acetate ($P \geq 0.115$) and propionate ($P \geq 0.266$) concentrations (mmol/L) were not different among treatment groups (Table 2.10). There was a diet x stage of supplementation interaction for butyrate concentrations ($P = 0.007$) which was explained by pigs receiving supplemental fiber at any point having increased butyrate concentrations, however pigs that received supplemental fiber only prior to weaning or after weaning had greater concentrations of butyrate when compared to pigs that received fiber for the entire study (Table 2.10). A diet by stage of supplementation interaction was observed for valerate concentrations. Pigs fed supplemental fiber pre-weaning but not post-weaning had greater concentrations of valerate compared to all other treatment groups ($P = 0.045$; Table 2.10) and pigs fed supplemental fiber for the entirety of the study had decreased fecal valerate concentrations. Pigs receiving supplemental fiber prior to weaning had decreased isobutyrate ($P = 0.050$) and a tendency for decreased isovalerate ($P = 0.058$) concentrations compared to other treatment groups (Table 2.10).

VFA data were analyzed as a concentration as well as a percent of total VFAs in the feces. When analyzed as a percentage, acetate accounted for a larger proportion of VFAs for pigs receiving supplemental fiber post-weaning ($P = 0.047$; Table 2.10). Propionate as a percentage of total VFAs did not differ among treatment groups ($P \geq 0.262$; Table 2.10). There was a diet by stage of supplementation interaction for butyrate percentages, with pigs being fed fiber only prior to weaning having a greater proportion of butyrate (as a percentage of total VFAs) than all other treatment groups ($P = 0.029$; Table 2.10). Pigs receiving supplemental fiber post-weaning had a decreased valerate as a percentage of total VFAs ($P = 0.038$; Table 2.10). Decreases in

isobutyrate ($P = 0.040$) and a tendency for isovalerate ($P = 0.051$), as a percentage of total VFAs was observed in pigs that received supplemental fiber pre-weaning compared to pigs that did not receive supplemental fiber pre-weaning (Table 2.10).

2.5 Discussion

This study was designed with the goal of alleviating the negative effects that are typically observed around weaning. These negative effects may occur from the stress involved with weaning, or due to decreased feed intake (Aherne et al., 1993). Prior to regulatory changes made in 2017, antibiotics were more commonly included in feed to prevent pathogens from weakening the immune system and causing intestinal distress, thus antibiotics increased weanling pig growth performance. As the need for animal protein sources for human consumption rises, so does the usage of antimicrobials in livestock production with the increased animal production to meet these demands. Antimicrobials however, pose a threat to human health with the potential rise of drug-resistant pathogens, which puts pressure on the swine industry to develop different means of lowering the use of antimicrobials through supplementation of various pathogen-suppressing feed additives (Van Boeckel et al., 2015).

Several researchers have reported the many benefits that are accompanied with adding fiber to swine diets (Burkitt, 1952; Cleave, 1968; Trowell, 1973; Perry and Ying, 2016). Fiber has been reported by Jenkins et al., (2002) to increase the concentration of short chain fatty acids (SCFA) which are capable of being used by the microbiota as an energy source. SCFA have been reported to boost the efficiency of the immune system by increasing the amount of natural killer cells (Pratt et al., 1986), and by making the cells less dependent on glutamine (Zhang et al., 1998) which then frees up glutamine to be used as an energy source by lymphocytes (Jenkins et al., 1999). This was observed in this study as well with increases in total SCFA production with

pigs fed dextrin. Most notable was the increase in butyrate production in pigs receiving supplemental fiber pre-weaning, and an even greater increase in pigs that received supplemental fiber solely pre- or post-weaning, which has been associated with reductions in inflammatory genes, increased growth performance, increased intestinal absorptive capacity, and decreases in E. *coli* (Lu et al., 2008). Fiber supplementation has also been reported to act as a prebiotic source in the gut which allows the growth of more beneficial bacteria (Bauer et al., 2006). Rossi et al., (2001) reported a reduction of E. *coli* in pigs, incubated with the infectious bacteria, following the inclusion of inulin into the diet. This same effect was noted in this study as well. A reduction in the number of bacteria associated with gut inflammation was observed, with a subsequent increase in the abundance of SCFA producing microbiota. Fiber consumption has also been associated with reduced inflammation within the digestive tract (King et al., 2007). This has even been reported on a cellular level in a study done by Baggio et al., (2019) who incubated TLR4 expressing cells with rhamnogalacturonan which consequently activated TLR4 reducing trans-epithelial resistance, thus reducing intestinal permeability. An increase in ileal and cecal GLP-2 expression was observed with fiber supplementation, which is associated with reduced intestinal inflammation and permeability (Burrin et al., 2003). Ileal occludin increased when supplemental fiber was fed prior to weaning indicating an improved gut barrier function (Oswald, 2006), but oddly it decreased when fiber was fed after weaning. HSP-70 expression also increased in pigs fed fiber post-weaning which may indicate that their intestines were better suited for intestinal reparation, since HSPs assist in cellular respiration (Bianchi, 2007), but it could also indicate that more were necessary to decrease inflammation that was already present. No changes in cecal occludin expression were observed, which may be explained by the fact that this line of pigs is

more highly susceptible to soy allergens, which may be due to a naturally more permeable intestinal barrier, making all treatment groups similar (Hashimoto-Hill et al., 2019).

Weaning is associated with reductions in villus height and increases in crypt depths (Cera et al., 1988). This is indicative of intestinal inflammation as well as increases in intestinal sloughing (Land, 2015). In this study, no changes in villi heights or villus height:crypt depth ratios were observed between treatment groups, though a trend for a reduction in crypt depths was observed with fiber supplementation pre-weaning. This implies that addition of fiber did not increase the absorptive capacity in the ileum but reduced the amount of stress placed on the small intestine (Nabuurs and Hoogendoorn, 1993). With a reduction in intestinal stress coupled with increased GLP-2 expression and SCFA production, growth performance would be expected to increase. However, when analyzed over the entire study, there were no differences in ADG or ADFI among treatment groups. The only growth performance data that showed any form of significance was G:F from day 11-21. This indicated pigs fed fiber prior to weaning were more efficient at this time, but this was not something that was observed over the course of the entire study.

Pigs that received supplemental fiber only prior to weaning or only post-weaning typically showed a more beneficial response than the pigs that received supplemental fiber for the entirety of the study. This was seen for response variables such as ileal and cecal GLP-2 expression, total VFA concentrations, acetate concentration, and butyrate concentration. These results were surprising as we expected pigs receiving supplemental fiber both pre- and post-weaning to have an increased response to these variables. This could be due to differences in carbohydrase production. Prior to weaning, lactase is the main carbohydrase being produced to digest the lactose in the sow's milk (Hartman et al., 1961). With the addition of dextrin, different

carbohydrases will be produced since dextrin is capable of being broken down in the small intestine due to the α-1,4 linkage (Takata et al., 2005; Singh et al., 2010). This could prepare the pig to better digest the multitude of carbohydrates that are available in solid feed after weaning. Pigs fed supplemental fiber prior to weaning could then be expected to perform better than those that had not received supplemental fiber prior to weaning. Pigs fed supplemental fiber for the entirety of the study then would digest more of the dextrin fed post-weaning, which limits the amount of it that would make it to the cecum to be fermented. Pigs being fed fiber only post-weaning would be expected to not have as high of carbohydrase production to break dextrin down in the small intestine which would allow more dextrin to make it to the cecum where it could be fermented by the bacteria to produce more SCFAs. This could explain the increases in the response variables that we observed for pigs that only received supplemental fiber either pre- or post-weaning.

2.6 Implications

Data from this study indicates that feeding supplemental soluble fiber prior to and/or after weaning resulted in changes in SCFA production, crypt depth, intestinal gene expression, and the cecal and colon microbiome. There were some benefits from feeding dextrin to pigs around weaning. To increase SCFA production, it may be beneficial to supplement fiber to pigs post-weaning. Including it prior to weaning is possible but proves to be more difficult while they are nursing. Though some beneficial effects from the addition of supplemental fiber were observed, the effects tended to be smaller than expected. Different types and sources of fiber have been reported to produce different effects in pigs. More work on how different fibers benefit the pig at weaning are warranted.

2.8 Literature Cited

Aherne, F., M. Hogberg, and H. Jones. 1993. Management and Nutrition of the Newly Weaned Pig. In: Pork Industry Handbook

Azman, S., A. F. Khadem, J. B. van Lier, G. Zeeman, and C. M. Plugge. 2015. Presence and Role of Anaerobic Hydrolytic Microbes in Conversion of Lignocellulosic Biomass for Biogas Production. Crit. Rev. Environ. Sci. Technol. 45:2523–2564. doi:10.1080/10643389.2015.1053727.

Baggio, C. H., J. Shang, A. M. Nascimento, T. R. Cipriani, M. Stephens, P. von der Weid, and W. MacNaughton. 2019. Dietary fibre improves intestinal epithelial barrier function through TLR4 activation. J. Can. Assoc. Gastroenterol. 2:147–148. doi:10.1093/jcag/gwz006.072

Bauer, E., B. A. Williams, and M. W. A. Verstegen. 2006. Fermentable carbohydrates: potential dietary modulators of intestinal physiology, microbiology and immunity in pigs. Biol. Grow. Anim. 4:33–63. doi:10.1016/S1877-1823(09)70089-X.

Bianchi, M. E. 2007. DAMPs, PAMPs and alarmins: all we need to know about danger. J. Leukoc. Biol. 81:1–5. doi:10.1189/jlb.0306164.

Burkitt, D. P. 1952. Acute abdomens--British and Baganda compared. East Afr. Med. J. 29:189–94.

Cabrix, R, A. Schinckel, T. Stewart, E. Herman, R Helm, and N. Nielsen. (2011) Development of tools to study immune-mediated allergenic responses to food and feed. In; Designing Soybeans for 21st Century Markets. Ed. R.Wilson. AOCS Press. pp 239-252

Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4:19. doi:10.1186/2049-1891-4-19.

Cera, K. R., D. C. Mahan, and R. F. Cross. 1988. Effect of Age , Weaning, and Postweaning Diet on Small Intestinal Growth and Jejunal Morphology in Young Swine 1 ' 2. 574–584.

Chen, H., X. Mao, J. He, B. Yu, Z. Huang, J. Yu, P. Zheng, and D. Chen. 2013. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets. Br. J. Nutr. 110:1837–1848. doi:10.1017/S0007114513001293.

Ciacci, C., Y. R. Mahida, A. Dignass, M. Koizumi, and D. K. Podolsky. 1993. Functional interleukin-2 receptors on intestinal epithelial cells. J. Clin. Invest. 92:527–532. doi:10.1172/JCI116598.

Clarke, L. C., T. Sweeney, E. Curley, S. K. Duffy, S. Vigors, G. Rajauria, and J. V. O'Doherty. 2018. Mycotoxin binder increases growth performance, nutrient digestibility and digestive health of finisher pigs offered wheat based diets grown under different agronomical conditions. Anim. Feed Sci. Technol. 240:52–65. doi:10.1016/j.anifeedsci.2018.03.013.

Cleave, T. L. 1968. Sucrose intake and coronary heart-disease. Lancet. 292:1187. doi:10.1016/S0140-6736(68)91659-0

Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucleic Acids Research. 2013 Dec 28;42(D1):D633–42

Dignass, A. U., and D. K. Podolsky. 1996. Interleukin 2 Modulates Intestinal Epithelial Cell Function in Vitro. Exp. Cell Res. 225:422–429. doi:10.1006/excr.1996.0193.

Fairbrother, J. M., É. Nadeau, and C. L. Gyles. 2005. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. Anim. Heal. Res. Rev. 6:17–39. doi:10.1079/AHR2005105.

Figliuolo, V. R., L. M. dos Santos, A. Abalo, H. Nanini, A. Santos, N. M. Brittes, C. Bernardazzi, H. S. P. de Souza, L. Q. Vieira, R. Coutinho-Silva, and C. M. L. M. Coutinho. 2017. Sulfate-reducing bacteria stimulate gut immune responses and contribute to inflammation in experimental colitis. Life Sci. 189:29–38. doi:10.1016/j.lfs.2017.09.014.

Fouhse, J. M., R. T. Zijlstra, and B. P. Willing. 2016. The role of gut microbiota in the health and disease of pigs. Anim. Front. 6:30–36. doi:10.2527/af.2016-0031.

Franklin, M. A., A. G. Mathew, J. R. Vickers, and R. A. Clift. 2002. Characterization of microbial populations and volatile fatty acid concentrations in the jejunum, ileum, and cecum of pigs weaned at 17 vs 24 days of age. J. Anim. Sci. 80:2904–2910. doi:10.2527/2002.80112904x.

Hampson, D. J. 1986. Alterations in piglet small intestinal structure at weaning. Res. Vet. Sci. 40:32–40. doi:10.1016/S0034-5288(18)30482-X.

Hartman, P. A., V. W. Hays, R. O. Baker, L. H. Neagle, and D. V. Catron. 1961. Digestive Enzyme Development in the Young Pig. J. Anim. Sci. 20:114–123. doi:10.2527/jas1961.201114x.

Hashimoto-Hill S, Kim M, Friesen L, Ajuwon KM, Herman E, Schinckel A, Kim CH. 2019. Differential food protein-induced inflammatory responses in swine lines selected for reactivity to soy antigens. Allergy.

Heo, J. M., F. O. Opapeju, J. R. Pluske, J. C. Kim, D. J. Hampson, and C. M. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J. Anim. Physiol. Anim. Nutr. (Berl). 97:207–237. doi:10.1111/j.1439-0396.2012.01284.x.

Heyman, M. 2005. Gut barrier dysfunction in food allergy. Eur. J. Gastroenterol. Hepatol. 17:1279–1285. doi:10.1097/00042737-200512000-00003.

Hötzel, M. J., G. P. P. de Souza, O. A. D. Costa, and L. C. P. Machado Filho. 2011. Disentangling the effects of weaning stressors on piglets' behaviour and feed intake: Changing the housing and social environment. Appl. Anim. Behav. Sci. 135:44–50. doi:10.1016/J.APPLANIM.2011.09.003.

Jenkins, D. J. A., C. W. C. Kendall, and V. Vuksan. 1999. Inulin, Oligofructose and Intestinal Function. J. Nutr. 129:1431S–1433S. doi:10.1093/jn/129.7.1431S

Jenkins, D. J. A., C. W. C. Kendall, L. S. A. Augustin, M. C. Martini, M. Axelsen, D. Faulkner, E. Vidgen, T. Parker, H. Lau, P. W. Connelly, J. Teitel, W. Singer, A. C. Vandenbroucke, L. A. Leiter, and R. G. Josse. 2002. Effect of wheat bran on glycemic control and risk factors for cardiovascular disease in type 2 diabetes. Diabetes Care. 25:1522–8. doi:10.2337/DIACARE.25.9.1522

Jung, B.-G., J.-A. Lee, and B.-J. Lee. 2012. Oxygenated Drinking Water Enhances Immune Activity in Pigs and Increases Immune Responses of Pigs during Salmonella Typhimurium Infection. J. Vet. Med. Sci. 74:1651–1655. doi:10.1292/jvms.12-0051.

Kelly, D., J. A. Smyth, and K. J. Mccracken. 1991. Digestive development of the early-weaned pig. Br. J. Nutr. 65:181. doi:10.1079/BJN19910079.

Khoury, D. El, C. Cuda, B. L. Luhovyy, and G. H. Anderson. 2012. Beta Glucan: Health Benefits in Obesity and Metabolic Syndrome. J. Nutr. Metab. 2012. doi:10.1155/2012/851362

King, D. E., B. M. Egan, R. F. Woolson, A. G. Mainous, Y. Al-Solaiman, and A. Jesri. 2007. Effect of a High-Fiber Diet vs a Fiber-Supplemented Diet on C-Reactive Protein Level. Arch. Intern. Med. 167:502–506. doi:10.1001/archinte.167.5.502

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology. 2013 Sep;79(17):5112–20.

Lallès, J.-P., G. Boudry, C. Favier, N. Le Floc'h, I. Luron, L. Montagne, I. P. Oswald, S. Pié, C. Piel, and B. Sève. 2004. Gut function and dysfunction in young pigs: physiology. Anim. Res. 53:301–316. doi:10.1051/animres:2004018.

Lallès, J.P., P. Bosi, H. Smidt, and C. R. Stokes. 2007. Weaning — A challenge to gut physiologists. Livest. Sci. 108:82–93. doi:10.1016/j.livsci.2007.01.091.

Land, W.G. 2015. The Role of Damage-Associated Molecular Patterns (DAMPs) in human diseases Part II: DAMPs as diagnostics, prognostics and therapeutics in clinical medicine. Sultan Qaboos University Medical Journal. 15(2):e157-e170

Li, H. M., T. Niki, T. Taira, S. M. M. Iguchi-Ariga, and H. Ariga. 2005. Association of DJ-1 with chaperones and enhanced association and colocalization with mitochondrial Hsp70 by oxidative stress. Free Radic. Res. 39:1091–1099. doi:10.1080/10715760500260348.

Lu, J., X. Zou, and Y. Wang. 2008. Effects of sodium butyrate on the growth performance, intestinal microflora and morphology of weanling pigs. J. Anim. Feed Sci. 17:568–578. doi:10.22358/jafs/66685/2008.

McKay, D. M., and A. W. Baird. 1999. Cytokine regulation of epithelial permeability and ion transport. Gut. 44:283–289. doi:10.1136/gut.44.2.283.

Moeser, A. J., C. Vander Klok, K. A. Ryan, J. G. Wooten, D. Little, V. L. Cook, and A. T. Blikslager. 2007. Stress signaling pathways activated by weaning mediate intestinal dysfunction in the pig. Am. J. Physiol. Liver Physiol. 292:G173–G181. doi:10.1152/ajpgi.00197.2006.

Nabuurs, M. J. A., A. Hoogendoorn, E. J. Van Der Molen, and A. L. M. Van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. Res. Vet. Sci. 55:78–84. doi:10.1016/0034-5288(93)90038-H.

Oswald, I. P. 2006. Role of intestinal epithelial cells in the innate immune defence of the pig intestine. Vet. Res. 37:359–368. doi:10.1051/vetres:2006006.

Pearce, S. C., M.-V. Sanz Fernandez, J. Torrison, M. E. Wilson, L. H. Baumgard, and N. K. Gabler. 2015. Dietary organic zinc attenuates heat stress–induced changes in pig intestinal integrity and metabolism12. J. Anim. Sci. 93:4702–4713. doi:10.2527/jas.2015-9018.

Perry, J. R., and Ying, W.. 2016. A Review of Physiological Effects of Soluble and Insoluble Dietary Fibers. J. Nutr. Food Sci. 6. doi:10.4172/2155-9600.1000476

Pilon, N., I. Daneau, V. Paradis, F. Hamel, J. G. Lussier, R. S. Viger, and D. W. Silversides. 2003. Porcine SRY Promoter Is a Target for Steroidogenic Factor 1. Biol. Reprod. 68:1098–1106. doi:10.1095/biolreprod.102.010884.

Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci. 62:131–144. doi:10.1017/S1357729800014417.

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51:215–236. doi:10.1016/S0301-6226(97)00057-2.

Pratt, V. C., K. A. Tappenden, M. I. McBurney, and C. J. Field. 1996. Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition Improves Nonspecific Immunity After Intestinal Resection in Rats. J. Parenter. Enter. Nutr. 20:264–271. doi:10.1177/0148607196020004264

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research. 2012 Dec 18;41(D1):D590–6.

R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2013.

Rossi, F., E. Cox, B. Goddeeris, D. Portetelle, J. Wavreille, and A. Thewis. 2001. Inulin incorporation in the weaned pig diet: intestinal coliform interaction and effect on specific systemic immunity. In: J. E. Lindberg and B. Ogle, editors. Digestive Physiology of Pigs : Proceedings of the 8th Symposium. CABI Pub, New York. p. 299–301

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and Environmental Microbiology. 2009 Dec;75(23):7537–41.

Singh, J., A. Dartois, and L. Kaur. 2010. Starch digestibility in food matrix: a review. Trends Food Sci. Technol. 21:168–180. doi:10.1016/J.TIFS.2009.12.001.

Takata, H., I. Kojima, N. Taji, Y. Suzuki, and M. Yamamoto. 2005. Industrial production of branching enzyme, and its application for producing a highly branched cyclic dextrin, Cluster Dextrin. 한국생물공학회 학술대회. 10:119–120. Trowell, H. 1973. Dietary fibre, ischaemic heart disease and diabetes mellitus. Proc. Nutr. Soc. 32:151–157. doi:10.1079/PNS19730033

Turner, J. R. 2006. Molecular Basis of Epithelial Barrier Regulation. Am. J. Pathol. 169:1901–1909. doi:10.2353/ajpath.2006.060681.

Ulger Toprak, N., T. Bozan, Y. Birkan, S. Isbir, and G. Soyletir. 2015. Butyricimonas virosa: the first clinical case of bacteraemia. New Microbes New Infect. 4:7–8. doi:10.1016/J.NMNI.2014.12.004.

Van Boeckel, T. P., C. Brower, M. Gilbert, B. T. Grenfell, S. A. Levin, T. P. Robinson, A. Teillant, and R. Laxminarayan. 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. 112:5649–5654. doi:10.1073/pnas.1503141112.

Verstegen, M. W. A., and B. A. Williams. 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. Anim. Biotechnol. 13:113–127. doi:10.1081/ABIO-120005774.

Wang, X., and G. R. Gibson. 1993. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J. Appl. Bacteriol. 75:373–380. doi:10.1111/j.1365-2672.1993.tb02790.x.

Wijtten, P. J. A., J. Van Der Meulen, and M. W. A. Verstegen. 2011. Intestinal barrier function and absorption in pigs after weaning: A review. Br. J. Nutr. 105:967–981. doi:10.1017/S0007114510005660.

Wilfinger, W. W., K. Mackey, and P. Chomczynski. 1997. Effect of pH and Ionic Strength on the Spectrophotometric Assessment of Nucleic Acid Purity. Biotechniques. 22:474–481.

Zhang, J., G. Wu, R. S. Chapkin, and J. R. Lupton. 1998. Energy Metabolism of Rat Colonocytes Changes during the Tumorigenic Process and Is Dependent on Diet and Carcinogen. J. Nutr. 128:1262–1269. doi:10.1093/jn/128.8.1262

Zhong, Y., M. Nyman, and F. Fåk. 2015. Modulation of gut microbiota in rats fed high-fat diets by processing whole-grain barley to barley malt. Mol. Nutr. Food Res. 59:2066–2076. doi:10.1002/mnfr.201500187.

Table 2.1 Composition of diets

	Phase 1		Phase 2		Phase 3	
Ingredient, %	Control	Treatment	Control	Treatment	Control	Treatment
Corn, yellow dent	35.71	35.71	40.57	39.57	45.69	44.69
Soybean meal, 47.5% CP	13.5	13.5	18.00	18.00	23.00	23.00
Soy protein concentrate	3.12	3.12	2.50	2.50	3.00	3.00
Soybean oil	3.62	3.62	4.00	4.00	1.66	1.66
Corn DDGS, 7%	0.00	0.00	0.00	0.00	10.00	10.00
Plasma, spray-dried	6.5	6.5	2.50	2.50	0.00	0.00
Selenium premix	0.05	0.05	0.05	0.05	0.05	0.05
Blood meal, spray-dried	1.00	1.00	1.00	1.00	0.00	0.00
Whey, dried	25.00	25.00	25.00	25.00	10.00	10.00
Fish meal, menhaden	4.00	4.00	4.00	4.00	4.00	4.00
Lactose	5.00	5.00	0.00	0.00	0.00	0.00
Limestone	1.17	1.17	0.94	0.94	0.93	0.93
Monocalcium Phosphate 21%	0.11	0.11	0.21	0.21	0.21	0.21
Trace Mineral premix[1]	0.13	0.13	0.15	0.15	0.15	0.15
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.47	0.47
Natuphos 600[3]	0.10	0.10	0.10	0.10	0.10	0.10
L-lysine-HCl	0.28	0.28	0.26	0.26	0.28	0.28
L-threonine	0.04	0.04	0.04	0.04	0.03	0.03
DL-methionine	0.18	0.18	0.15	0.15	0.15	0.15
L-tryptophan	0.00	0.00	0.03	0.03	0.03	0.03
Fiber premix[4]	0.00	0.00	0.00	1.00	0.00	1.00
ME, Kcal/lb	1594	1594	1592	1577	1543	1527
Crude Protein, %	23.26	23.26	22.27	22.19	23.57	23.49
Lysine, %	1.79	1.79	1.62	1.62	1.54	1.53
SID Lysine, %	1.64	1.64	1.48	1.48	1.37	1.37
SID Threonine, %	0.96	0.96	0.87	0.87	0.81	0.81
SID Tryptophan, %	0.28	0.28	0.28	0.28	0.26	0.26
SID Methionine, %	0.50	0.50	0.47	0.47	0.47	0.46
SID Methionine + Cysteine, %	0.90	0.90	0.81	0.81	0.76	0.76
SID Valine, %	1.07	1.07	0.98	0.97	0.97	0.96
SID Isoleucine, %	0.84	0.84	0.82	0.82	0.87	0.86
SID Leucine, %	1.88	1.88	1.76	1.75	1.58	1.57
Calcium, %	0.94	0.94	0.88	0.88	0.78	0.78
Phosphorous, %	0.64	0.64	0.64	0.64	0.63	0.62
Available Phosphorous, %	0.43	0.43	0.42	0.42	0.35	0.35

[1]Trace mineral premix provided the following guaranteed minimums per kg diet: iron, 84.7 mg; zinc, 84.7 mg; manganese, 10.5 mg; copper, 7.87 mg; iodine, 0.32 mg.

[2]Vitmain premix provided the following guaranteed minimums per kg diet: vitamin A, 9000 IU; vitamin E, 187 IU; vitamin K (hetrazeen), 2.62 mg; vitamin B_1, 1.857 mg; vitamin B_{12}, 17.25 µg; riboflavin, 5.25 mg; d-pantothenic acid, 11.25 mg;
niacin, 18.75 mg.

[3]Contains 600 U/g of phytase activity

Table 2.2 Analyzed composition of diets

	Phase 1	Phase 2		Phase 3	
Nutrient[1]		No Fiber	Fiber	No Fiber	Fiber
Nitrogen, %	3.81	3.43	3.67	3.54	3.49
Metabolizable Energy, Kcal/kg	4152	3506	4160	4037	4019
Dry Matter, %	88.42	86.97	87.75	86.8	87.25
Ash, %	5.2	5.37	5.27	4.67	4.6
Phosphorous, %	0.667	0.703	0.685	0.739	0.628
NDF, %	1.83	5.15	3.95	10.26	13.29
ADF, %	-0.765	1.86	0.114	3.12	5.73

[1] Analyzed at Purdue University

Table 2.3 Primer Sequences used for Intestinal Gene Expression.

Gene	Sense (5'-3') – Forward	Antisense (5'-3') - Reverse	References
GLP-2	TCCCGGTGCTCTTTGTTGTC	TACCCAGCACCCTGTGTTCTC	Clarke et al., 2018
HSP 70	GCCCTGAATCCGCAGAATA	TCCCCACGGTAGGAAACG	Pilon et al., 2003
Occludin	TCGTCCAACGGGAAAGTGAA	ATCAGTGGAAGTTCCTGAACCA	Pearce et al., 2015
GAPDH	GAAGGTCGGAGTGAACGGAT	CATGGGTAGAATCATACTGGAACA	Li et al., 2005

Table 2.4 Effects of soluble fiber supplementation prior to weaning on growth performance, histology, and gene expression.

Treatment	No Fiber	Fiber	SE	Probability, P<
BW d-14, pigs killed d0 (n=8)	2.89	2.53	0.369	0.525
BW d-14, all pigs (n=40)	3.19	3.12	0.089	0.587
BW d-3, pigs killed d0	5.32	5.25	0.831	0.956
BW d-3, all pigs	5.51	5.55	0.101	0.771
BW d0, pigs killed d0	6.09	5.90	0.942	0.890
BW d0, all pigs	6.15	6.20	0.122	0.778
ADG day -14 – 0, pigs killed d0	0.229	0.240	0.048	0.872
ADG day -14 – 0, all pigs	0.211	0.219	0.009	0.510
Pigs Harvested d0 (n=8)				
Villus Height, μm	348	311	26.4	0.355
Crypt Depth, μm	168	157	11.6	0.526
Villus Height:Crypt Depth	2.07	1.98	0.106	0.580
Ileal GLP-2, relative fold change	17.21	7.78	7.40	0.402
Ileal Occludin, relative fold change	2.84	2.63	1.37	0.920
Cecal GLP-2, relative fold change	2.93	1.24	1.18	0.367
Cecal HSP-70, relative fold change	3.57	2.14	1.83	0.587
Cecal Occludin, relative fold change	3.47	1.86	1.55	0.504

Table 2.5 Effect of soluble fiber supplementation post-weaning on growth and development.

Pre-Weaning Fiber	No	No	Yes	Yes				
Post-Weaning Fiber	No	Yes	No	Yes		Probability, P<		
Number of pigs after weaning	8	7	8	8	SE	Pre-wean Main Effect	Post-wean Main Effect	Diet x Stage
Day 0 - 4								
ADG, kg/d	0.108	0.137	0.086	0.070	0.043	0.261	0.873	0.571
ADFI, kg/d	0.139	0.165	0.132	0.119				
d 4 Wt, kg	6.70	6.38	6.47	6.61	0.216	0.984	0.643	0.256
Day 4 - 11								
ADG, kg/d	0.185	0.123	0.130	0.103	0.057	0.474	0.402	0.737
ADFI, kg/d	0.271	0.261	0.294	0.225	0.046	0.873	0.351	0.497
G:F	0.538	0.210	0.344	0.286	0.170	0.706	0.227	0.395
d 11 Wt, kg	8.00	7.24	7.38	7.33	0.503	0.574	0.389	0.448
Day 11- 21								
ADG, kg/d	0.261	0.348	0.363	0.359	0.045	0.208	0.337	0.304
ADFI, kg/d	0.486	0.612	0.562	0.529	0.071	0.956	0.491	0.253
G:F	0.528	0.541	0.655	0.672	0.052	0.018	0.763	0.968
d 21 Wt, kg	10.7	10.9	11.1	10.3	0.910	0.921	0.697	0.566
Overall Post-Weaning Day 4 - 21								
ADG, kg/d	0.235	0.290	0.272	0.215	0.057	0.698	0.977	0.262
ADFI, kg/d	0.402	0.485	0.455	0.376	0.068	0.643	0.970	0.182
G:F	0.568	0.573	0.599	0.521	0.072	0.868	0.557	0.501

Table 2.6 Effects of soluble fiber supplementation prior to weaning on villus height, crypt depth, and villus height:crypt depth for pigs euthanized on d21.

Pre-weaning Fiber	No	Yes	SE	Probability, P<
Villus Height, μm	387	393	15.22	0.781
Crypt depth, μm	342	309	14.81	0.097
Villus Height:Crypt Depth	1.14	1.28	0.065	0.117

Table 2.7 Effects of soluble fiber supplementation post-weaning on villus height, crypt depth, and villus height:crypt depth on d 21 post-weaning.

Post-weaning Fiber	No	Yes	SE	Probability, P<
Villus Height, μm	391	389	14.58	0.932
Crypt depth, μm	338	313	14.18	0.198
Villus Height:Crypt Depth	1.25	1.17	0.062	0.365

Table 2.8 Interactive effects of fiber supplementation pre- and post-weaning on villus height, crypt depth, and villus height:crypt depth on d 21 post-weaning

Fiber pre-weaning	No	No	Yes	Yes		Probability, P<		
Fiber post-weaning	No	Yes	No	Yes	SE	Pre-wean	Post-wean	Diet x Stage
Villus Height, μm	377	405	398	381	21.4	0.781	0.932	0.265
Crypt depth, μm	358	319	327	299	20.8	0.097	0.198	0.775
Villus Height:Crypt Depth	1.08	1.27	1.21	1.29	0.091	0.117	0.365	0.479

Table 2.9 Effects of soluble fiber supplementation either pre-weaning and/or post weaning on relative abundance of a specified bacterial genus

	Relative Abundance in Cecum		Relative Abundance in Large Intestine	
Bacterial Genus	Fiber	No Fiber	Fiber	No Fiber
Butyrivibrio	0.000451	0		
Butyricimonas	0.000761	0		
Acetivibrio	0.002366	0	0.007606	0
Turicibacter	0.000263	0	0.000423	0
Desulfovibrionaceae unclassified	0	0.000254	0	0.000676
Desulfovibrionaceae bilophila	0.000282[4]	0	0.000254	0

[1]Analysis was done by Dr. Tim Johnson's lab at Purdue University
[2]Data represents pigs that received fiber at any given point versus pigs that did not receive fiber
[3]Blank spaces indicate no analysis was done in that organ
[4]This value only represents pigs that received fiber only prior to weaning

Table 2.10 Main and interaction effects of soluble fiber supplementation pre- and post-weaning on fecal VFA concentrations and percentages

Pre-Weaning Fiber	No	No	Yes	Yes				
Post-Weaning Fiber	No	Yes	No	Yes		Probability, P<		
Number of pigs after weaning	8	7	8	8	SE	Pre-wean Main Effect	Post-wean Main Effect	Diet x Stage
Total VFA's, mmol/L	144	182	180	164	14.0	0.507	0.401	0.054
Acetate, mmol/L	73	96	87	86	7.68	0.773	0.141	0.115
Acetate, % of total	51	52	48	53	1.54	0.595	0.047	0.216
Propionate, mmol/L	38	47	44	43	4.84	0.830	0.452	0.266
Propionate, % of total	26	25	25	26	1.06	0.486	0.966	0.262
Butyrate, mmol/L	24	30	37	28	2.70	0.057	0.497	0.007
Butyrate, % of total	17	17	21	16	1.02	0.070	0.047	0.029
Valerate, mmol/L	6.07	6.29	9.72	5.92	0.990	0.096	0.072	0.045
Valerate, % of total	4.15	3.68	5.40	3.51	0.555	0.314	0.038	0.190
Isobutyrate, mmol/L	1.23	1.43	0.884	0.829	0.237	0.050	0.746	0.571
Isobutyrate, % of total	0.873	0.923	0.503	0.497	0.190	0.040	0.905	0.878
Isovalerate, mmol/L	1.22	1.44	0.765	0.845	0.304	0.058	0.745	0.961
Isovalerate, % of total	0.953	0.988	0.438	0.504	0.253	0.051	0.832	0.949

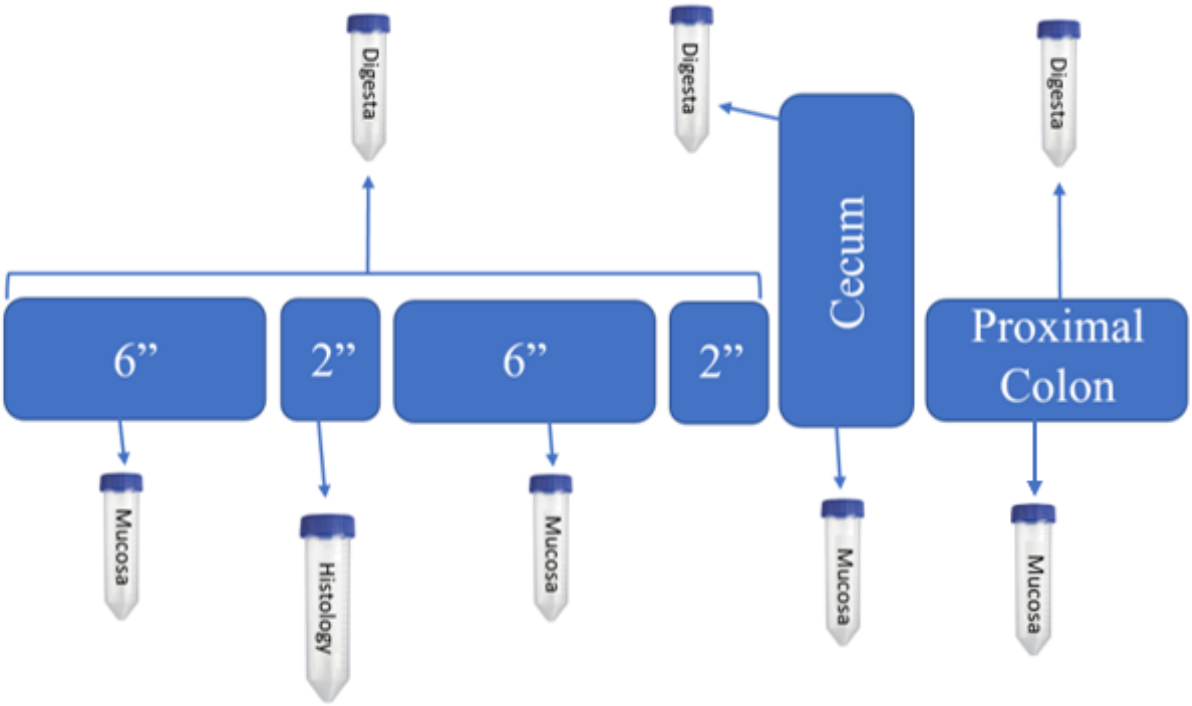


Figure 2.1 Sample collection locations within the pig ileum, cecum, and large intestine.

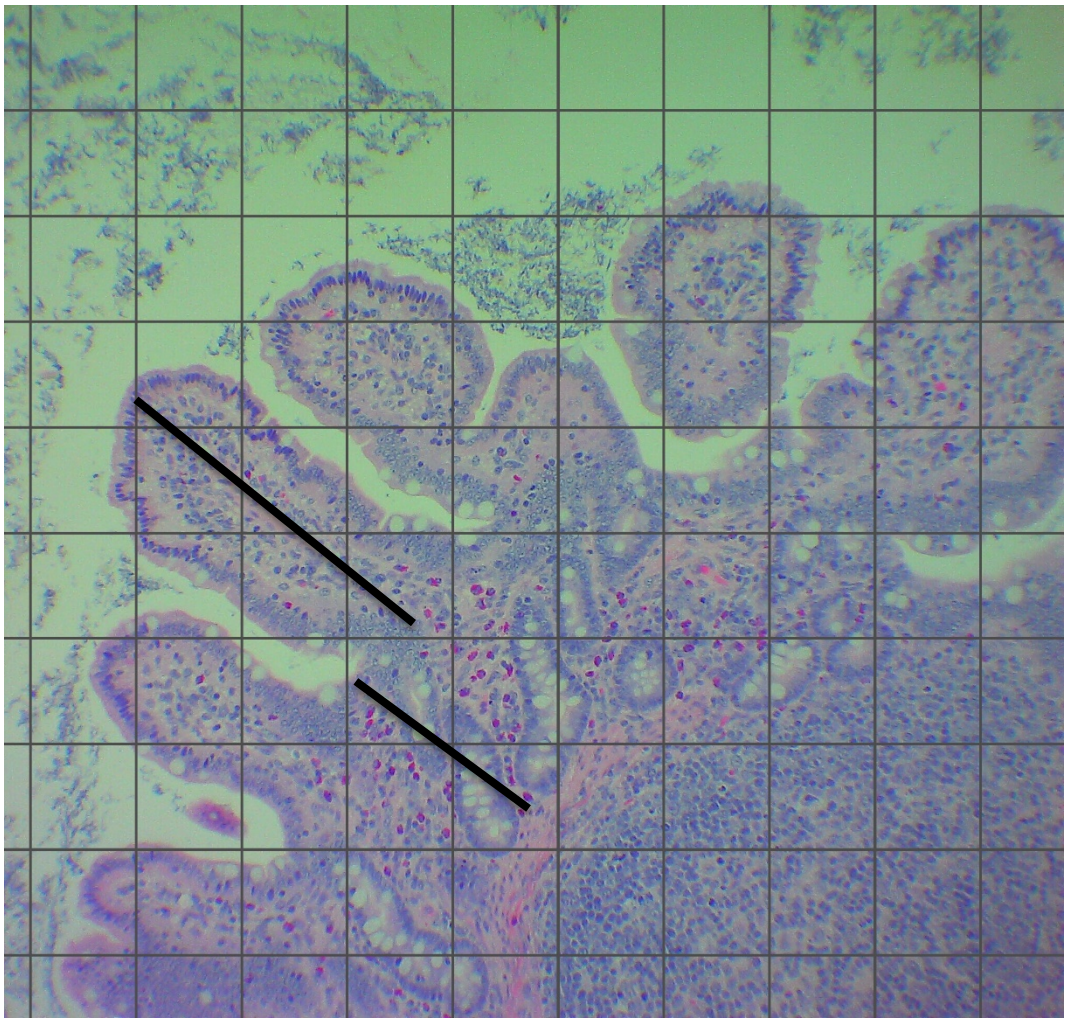


Figure 2.2 Histological cross section of ileum with lines drawn to represent measurements for villus height and crypt depth.

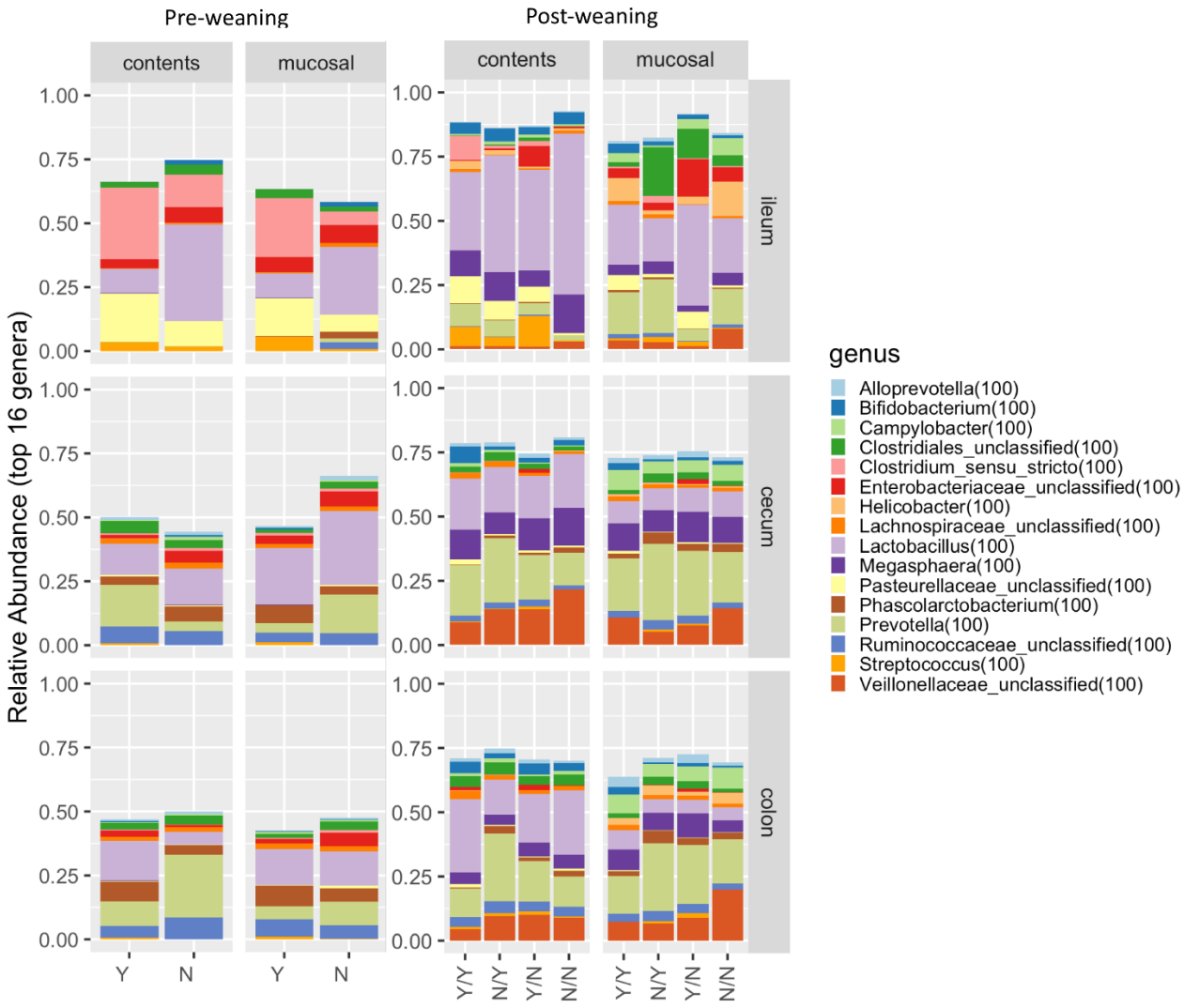


Figure 2.3 Effect of soluble fiber supplementation on top 16 genera present in intestinal microbiome.

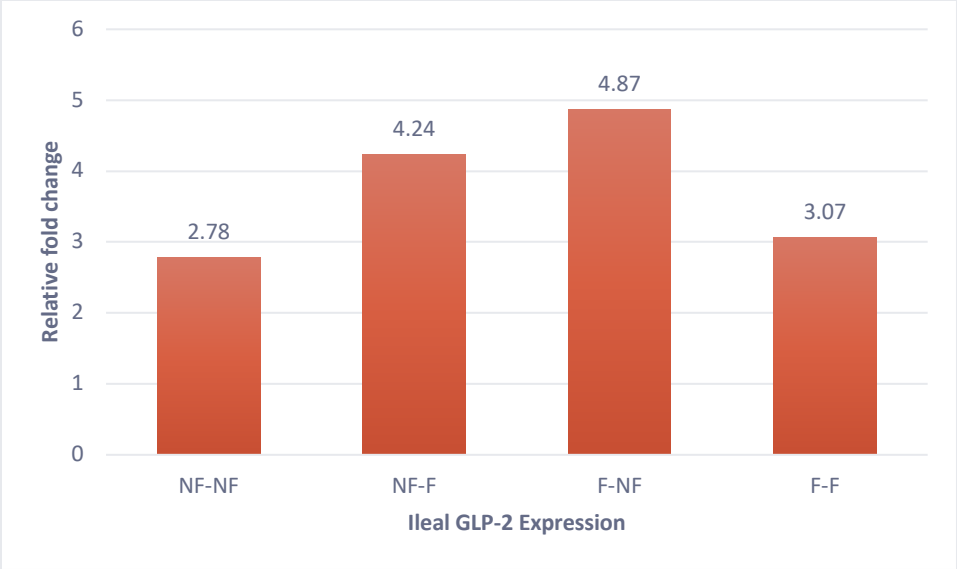


Figure 2.4 Effect of soluble fiber supplementation on Ileal GLP-2 gene expression analyzed by relative fold change d21 post-weaning.

Pre-weaning main effect ($P = 0.337$). Post-weaning main effect ($P = 0.725$). Diet x stage interaction effect ($P = 0.002$). NF-NF is control group that did not receive supplemental fiber either before or after weaning. F-NF only received supplemental fiber before weaning. NF-F received supplemental fiber only after weaning. F-F received supplemental fiber both before and after weaning. SE = 0.525

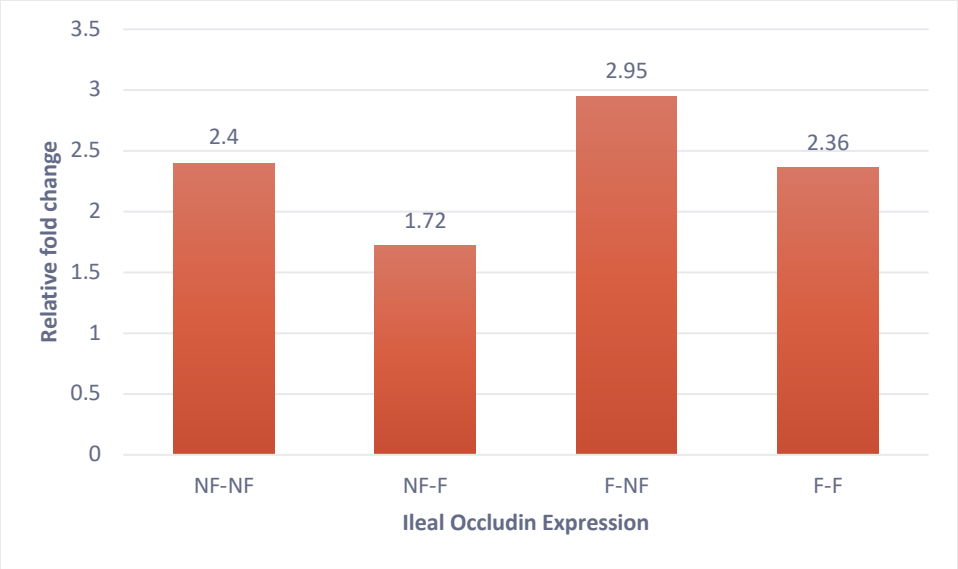


Figure 2.5 Effect of soluble fiber supplementation on Ileal occludin gene expression analyzed by relative fold change d21 post-weaning.

Pre-weaning main effect ($P = 0.086$). Post-weaning main effect ($P = 0.053$). Diet x stage interaction effect ($P = 0.890$). NF-NF is control group that did not receive supplemental fiber either before or after weaning. F-NF only received supplemental fiber before weaning. NF-F received supplemental fiber only after weaning. F-F received supplemental fiber both before and after weaning. SE = 0.346

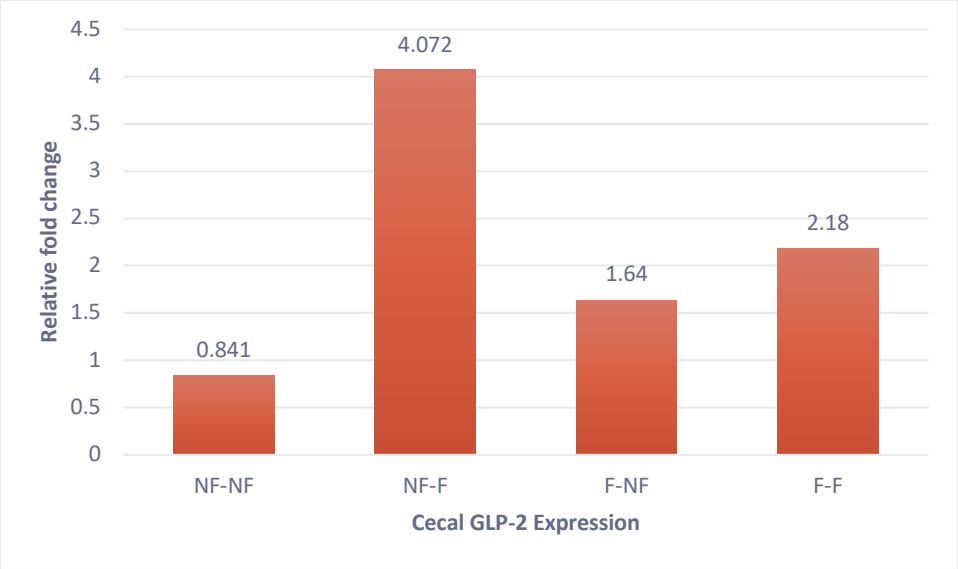


Figure 2.6 Effect of soluble fiber supplementation on cecal GLP-2 gene expression analyzed by relative fold change d21 post-weaning.

Pre-weaning main effect ($P = 0.338$). Post-weaning main effect ($P = 0.007$). Diet x stage interaction effect ($P = 0.049$). NF-NF is control group that did not receive supplemental fiber either before or after weaning. F-NF only received supplemental fiber before weaning. NF-F received supplemental fiber only after weaning. F-F received supplemental fiber both before and after weaning. SE = 0.689

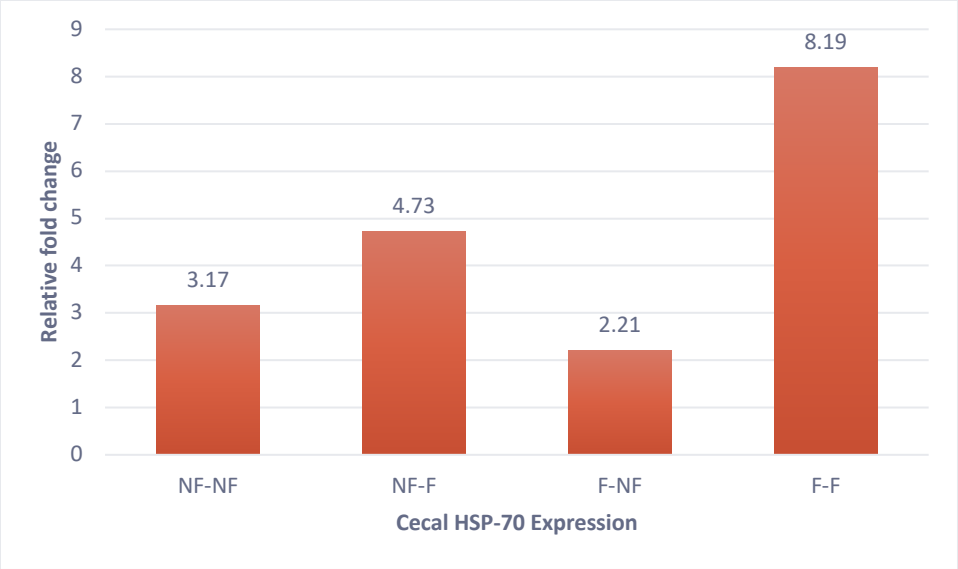


Figure 2.7 Effect of soluble fiber supplementation on cecal HSP-70 gene expression analyzed by relative fold change d21 post-weaning.

Pre-weaning main effect ($P = 0.388$). Post-weaning main effect ($P = 0.012$). Diet x stage interaction effect ($P = 0.148$). NF-NF is control group that did not receive supplemental fiber either before or after weaning. F-NF only received supplemental fiber before weaning. NF-F received supplemental fiber only after weaning. F-F received supplemental fiber both before and after weaning. SE = 1.59

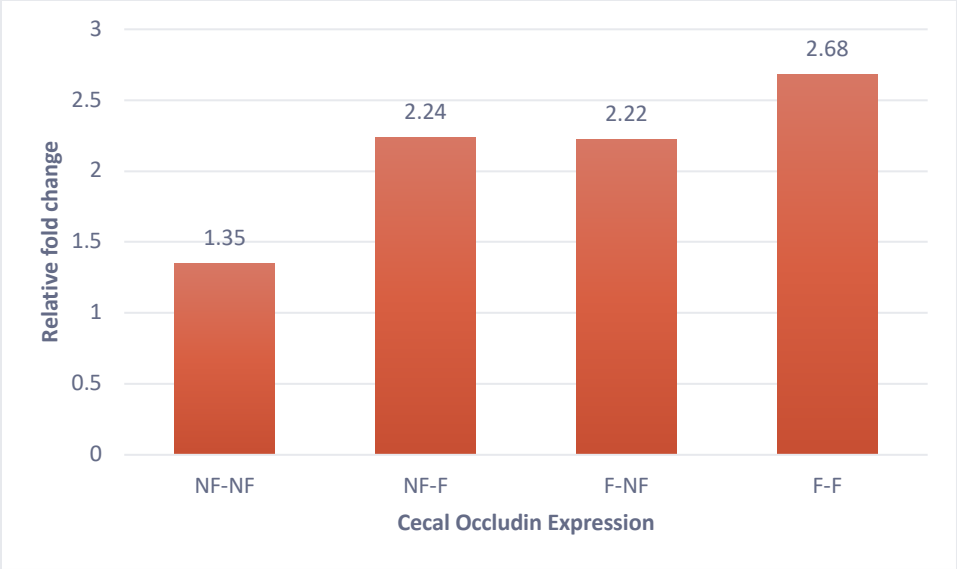


Figure 2.8 Effect of soluble fiber supplementation on cecal occludin gene expression analyzed by relative fold change d21 post-weaning.

Pre-weaning main effect (P = 0.132). Post-weaning main effect (P = 0.121). Diet x stage interaction effect (P = 0.619). NF-NF is control group that did not receive supplemental fiber either before or after weaning. F-NF only received supplemental fiber before weaning. NF-F received supplemental fiber only after weaning. F-F received supplemental fiber both before and after weaning. SE = 0.494

CHAPTER 3. CONCLUSIONS AND FUTURE DIRECTION

3.1 Conclusions

The objective of this study was to determine if supplementing soluble fiber into the diet of pigs, either before or after weaning, aids in eliminating some of the negative effects that commonly occur after weaning. Finding ways to increase health and growth of pigs post-weaning, which is one of the more susceptible times of their life, has been an important area of research for the swine industry. Chapter 2 of this thesis demonstrates that supplementing fiber at different times can alter the gut microbiome, volatile fatty acid (VFA) production, intestinal morphology, and intestinal gene expression.

Chapter 2 demonstrates that soluble fiber supplementation, either before and/or after weaning, can alter the intestinal microbiome. Bacteria that have been shown to produce short chain fatty acids (SCFA) increased in pigs that were fed soluble fiber at any point in the study. This indicates that fiber supplementation is able to increase SCFA producing bacteria which then increases the amount of SCFA's available to the pig. These SCFA's can then be used as a source of energy for enterocytes and improve gut health. An increase in bacteria associated with intestinal inflammation was also observed in pigs that never received supplemental fiber, which indicates increased intestinal permeability. However, one bacterial genus of the *Desulfovibrionacea* family, *bilophila,* had a higher concentration in pigs that only received supplemental fiber prior to weaning.

Total VFA concentrations were greater in pigs receiving supplemental fiber, with pigs only receiving supplemental soluble fiber prior to or after weaning having the highest concentrations. This proves to be beneficial to the pig's health as it provides a source of energy for the intestinal mucosa and increases the effectiveness of the immune system (Pratt et al., 1986; Jenkins et al.,

1999; Segain et al., 2000). Most notably, there were increased butyrate concentrations in some treatment groups that had received supplemental soluble fiber. Differences were observed in intestinal morphology as well. Fiber supplementation tended to cause pigs to have increased crypt depths when fed prior to weaning. This does not indicate increased absorptive capacity; however, the shorter crypts indicate less intestinal stress around the time of weaning, which is very crucial due to the low feed intake that typically occurs around weaning (Nabuurs and Hoogendoorn, 1993; Wijtten et al., 2011).

A few different changes were observed among treatment groups for the gene expression of proteins known to be associated with intestinal inflammation or intestinal recovery. In the ileum there was a difference among treatment groups for both GLP-2 and occludin gene expression, and in the cecum there was a difference among treatment groups for GLP-2 and HSP-70 gene expression. Pigs that received supplemental soluble fiber only after weaning had increased gene expression of GLP-2 in the cecum and the ileum, and pigs that received soluble fiber prior to weaning only had increased GLP-2 expression in the ileum, which indicated that these pigs were better suited to recover from intestinal inflammation. This increase in GLP-2 expression is usually indicative of a reduction in intestinal permeability and inflammation, and an increase in cell proliferation (Burrin et al.,2003). Pigs that received supplemental fiber only after weaning had the greatest cecal GLP-2 expression, almost over twice that of any other treatment group, and over 4 times higher than pigs that never received supplemental fiber at any point during the study. This could be due to the fact that more of the dextrin was able to reach the cecum for fermentation since the pigs have not been exposed to it before weaning and thus have not increased the development of carbohydrases that are capable of breaking down dextrin in the small intestine. GLP-2 is activated via a G-protein receptor located within the intestinal mucosa

and is released from intestinal L-cells (Burrin et al., 2003). The GLP-2 receptor does not localize in the enterocytes however, but in the endocrine cells which suggests that it is controlled through a secondary signaling system (Figure 3.1). Heat shock protein 70 is a chaperonin protein that aids in the recovery of the small intestine following a form of stress and is typically known to increase in pigs following weaning (Lallès et al., 2004; Bianchi, 2007). Pigs that received supplemental fiber after weaning had higher HSP-70 gene expression. This could be interpreted as if the stress of weaning was greater in pigs receiving fiber so an increased expression of HSP-70 was necessary for reparation, or this could be interpreted as if they were better suited for intestinal recovery with increased expression of the beneficial chaperonin protein, which seems more likely given the other effects observed. Occludin expression increased in the ileum in pigs that were fed fiber prior to weaning but decreased in pigs that were fed fiber after weaning. Pigs that only received fiber after weaning had a much lower occludin expression than the other treatment groups, which partially caused these significances to occur. The reason that this treatment group had much lower occludin expression is unknown. Cecal occludin expression was nearing a trend for pre-wean and post-wean main effects, with pigs being fed fiber having numerically greater levels of occludin expression though it was not quite significant or a trend. Due to weaning causing intestinal permeability and inflammation, a breakdown in tight junctions typically occurs. Occludin expression was expected to increase with fiber supplementation which occurred with supplementation prior to weaning but oddly not with supplementation after weaning. The reason for this is unknown, but due to the pigs having an increased responsiveness to the soy allergen β-conglycinin, intestinal inflammation may have been worsened by the presence of soybean meal in the feed and negated increased occludin expression to a certain degree.

3.2 Future Direction

Though we did see that adding supplemental soluble fiber in the form of dextrin to the diet of pigs around weaning ameliorated some negative effects that occur around weaning, we did not see all the effects that were expected. Some of the changes that did occur were of smaller magnitude than expected as well. This could partly be due to the ability of the pig's body being able break down dextrin prior to cecal fermentation (Singh et al., 2010). More work needs to be done on testing different sources of fiber before and/or after weaning. Other fiber sources have been reported to cause increased growth rate and intestinal reparation when pigs have intestinal distress (Chen et al., 2013). This study also focused more on the inclusion of fiber at different time points, not on the ideal inclusion level of fiber in the diet, which could warrant future research. Some of the data shows that the pigs that either switched on or off fiber at weaning had a numerically increased beneficial response than the pigs that received supplemental fiber for the entirety of the study. This could indicate that the fiber inclusion level was too great, or that if fed the same fiber source for an extended period of time, the body is capable of breaking the fiber down earlier in the digestive tract, thus limiting the effect it has on the microbiota. Due to the varying effects of fiber supplementation from different sources, mixing different fiber sources, or periodical switching of fiber sources may prove to be beneficial and requires more research. This study looked at the effects of fiber supplementation around weaning, and some conclusions were drawn, but this study only scratched the surface of the many response variables that can indicate intestinal distress. For instance, more gene expression work could be performed as fiber supplementation may not have affected occludin expression, but it may have impacted other tight junction protein gene expression, or possibly the expression of other inflammatory markers. Different cytokines involved in the inflammatory or healing process should be studied to see if their expression is affected such as TNF-α, IL-2, IL-6, and IL-10. More work on other genetic

strains of pigs may be warranted as well, it is possible that the increased sensitivity to the soy allergy offset some of the intestinal recovery in the pigs, so testing the same fiber on pigs with less reactivity to soy proteins may yield different results.

3.4 Literature Cited

Bianchi, M. E. 2007. DAMPs, PAMPs and alarmins: all we need to know about danger. J. Leukoc. Biol. 81:1–5. doi:10.1189/jlb.0306164.

Burrin, D. G., B. Stoll, and X. Guan. 2003. Glucagon-like peptide 2 function in domestic animals. Domest. Anim. Endocrinol. 24:103–122. doi:10.1016/S0739-7240(02)00210-2.

Chen, H., X. Mao, J. He, B. Yu, Z. Huang, J. Yu, P. Zheng, and D. Chen. 2013. Dietary fibre affects intestinal mucosal barrier function and regulates intestinal bacteria in weaning piglets. Br. J. Nutr. 110:1837–1848. doi:10.1017/S0007114513001293.

Jenkins, D. J. A., C. W. C. Kendall, and V. Vuksan. 1999. Inulin, Oligofructose and Intestinal Function. J. Nutr. 129:1431S–1433S. doi:10.1093/jn/129.7.1431S.

Lallès, J.-P., G. Boudry, C. Favier, N. Le Floc'h, I. Luron, L. Montagne, I. P. Oswald, S. Pié, C. Piel, and B. Sève. 2004. Gut function and dysfunction in young pigs: physiology. Anim. Res. 53:301–316. doi:10.1051/animres:2004018.

Nabuurs, M. J. A., A. Hoogendoorn, E. J. Van Der Molen, and A. L. M. Van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. Res. Vet. Sci. 55:78–84. doi:10.1016/0034-5288(93)90038-H.

Pratt, V. C., K. A. Tappenden, M. I. McBurney, and C. J. Field. 1996. Short-Chain Fatty Acid-Supplemented Total Parenteral Nutrition Improves Nonspecific Immunity After Intestinal Resection in Rats. J. Parenter. Enter. Nutr. 20:264–271. doi:10.1177/0148607196020004264.

Segain, J.-P. 2000. Butyrate inhibits inflammatory responses through NFkappa B inhibition: implications for Crohn's disease. Gut. 47:397–403. doi:10.1136/gut.47.3.397.

Singh, J., A. Dartois, and L. Kaur. 2010. Starch digestibility in food matrix: a review. Trends Food Sci. Technol. 21:168–180. doi:10.1016/J.TIFS.2009.12.001.

Wijtten, P. J. A., J. Van Der Meulen, and M. W. A. Verstegen. 2011. Intestinal barrier function and absorption in pigs after weaning: A review. Br. J. Nutr. 105:967–981. doi:10.1017/S0007114510005660.

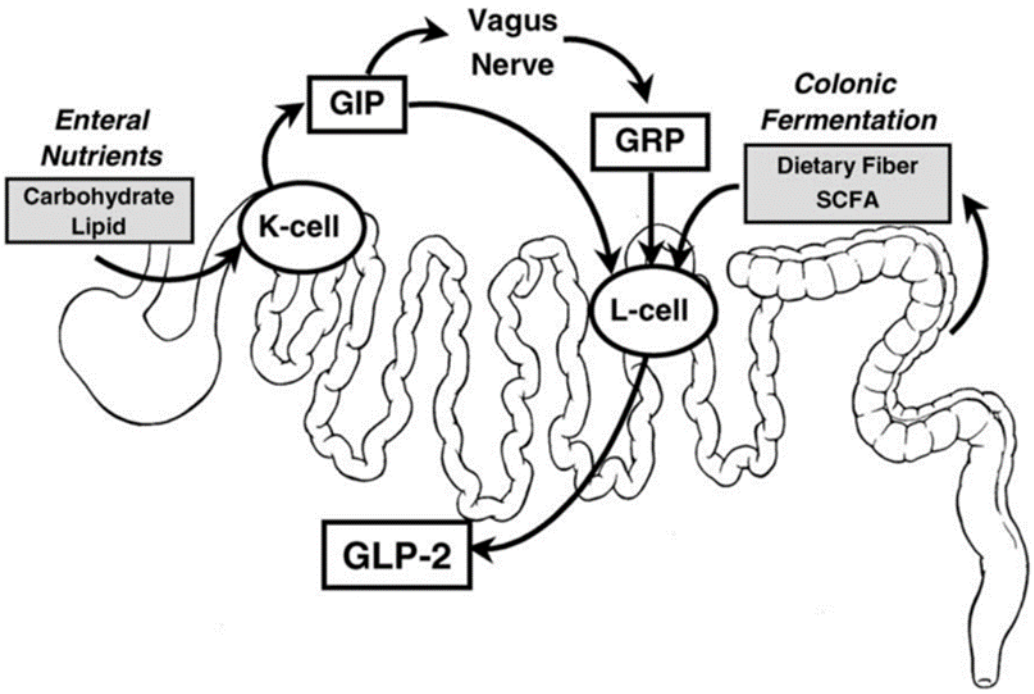


Figure 3.1 Schematic overview of factors affecting GLP-2 secretion (from Burrin et al., 2003)