UNDERSTANDING THE HEALTH BENEFITS OF FEEDING TREATED WHEAT STRAW TO WEANLING PIGS

By

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ABSTRACT

Dietary fiber (DF) is a promising low-cost feedstuff with the potential to support growth and enhance immune function in the young pig. Historically, fiber has been thought to be unfavorable for young pigs due to potential negative effects on energy density, growth rate, and feed intake. Recent research has challenged this perception of fiber and suggests DF can be beneficial for growth and health. The weanling pig may benefit from DF as it enhances gastric function, like motility and short-chain fatty acid production, leading to efficient nutrient utilization. Dietary intervention can offer a means to mitigate diseases by enhancing immune system function. Dietary fiber encompasses a range of carbohydrates and analytical methods based on physicochemical properties. These analytic methods have evolved over time to incorporate properties of fiber, such as solubility, viscosity, and water-holding capacity. Each impacts the weaned pig's growth and health. Processing fibrous by-products is a solution to enhance the digestibility and nutrient availability of these products for better utilization in monogastric animals. Treatment processes alter the character of fibrous components by depolymerizing hemicellulose and lignin, releasing an energy source, short-chain fatty acids. Treatment processes through hydrothermal, chemical, and pressure are crucial for countries that produce mass quantities of by-products instead of traditional grains that may be scarce. This thesis aims to explore the characteristics of DF and their biological roles in the growth, health, and well-being of weaned pigs. It seeks to address how treatment processes can modify fibrous characteristics to contribute to standard growth and health parameters.

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LIST OF ABBREVIATIONS

ADF = Acid detergent fiber

ADFI = Average daily feed intake

ADG = Average daily gain

AOAC = Association of Official Analytical Chemists

CF = Crude fiber

CON = Control

DF = Dietary fiber

G:F = Gain to feed ratio, feed efficiency

iFABP = Intestinal fatty acid-binding protein

FABP = Fatty acid-binding protein

IDF = Insoluble dietary fiber

IgA = Immunoglobulin A

IL-6 = Interleukin 6

IL-10 = Interleukin 10

IL-12 = Interleukin 12

NDF = Neutral detergent fiber

PCV2 = Porcine Circovirus Type 2

SCFA = Short-chain fatty acid

SDF = Soluble dietary fiber

TDF = Total dietary fiber

TNF-α = Tumor necrosis factor-alpha

TWS = Treated wheat straw

CHAPTER 1: LITERATURE REVIEW

Introduction

Dietary fiber (DF) is present in various low-cost feedstuffs found globally and has the potential to support growth and positively augment immune function when fed to young pigs (Bikker et al., 2006; Mateos et al., 2006; Weber et al., 2008). Weanling pigs experience stress as they transition from farrowing to the nursery environment. New diets, means of hydration, and social groups while they are growing and developing leave weanling pigs susceptible to enteric and other diseases. The inclusion of DF in the diet is historically thought unfavorable to young pigs, as it has been reported that increased DF leads to lower energy density, decreased growth rate, and feed intake (Bach Knudsen, 2001; Le Goff and Noblet, 2001; Hedemann et al., 2006). However, current literature reports growth performance need not be synergistic with DF, instead, DF may benefit performance (Liu et al., 2018; Chen et al., 2020). Weaned pigs may perform more efficiently because DF causes improved gastric functions like motility and short-chain fatty acid (SCFA) production allowing the animal to utilize nutrients more efficiently (Anugwa et al., 1989; Pond et al., 1989; Shi and Noblet, 1993; Zhao et al., 2018). Dietary intervention may be a way to mitigate disease by improving the overall function of the immune system determined by immune markers for intestinal integrity in relation to dietary treatment (Weber et al., 2008).

Dietary fiber is a broad term for carbohydrates found in plant-based foods and has different methods of analysis based on physicochemical properties. There are multiple ways to analyze fiber, but the predominate methods are crude fiber, total dietary fiber (TDF), neutral detergent fiber (NDF), and acid detergent fiber (ADF) when formulating diets. Dietary fiber can be characterized by solubility, viscosity, and water-holding capacity, each affecting gastric function (Schneeman, 2000). Solubility is separated into soluble or insoluble. Viscosity relates to physical

interactions in the intestine when the material encounters water. Water-holding capacity is fiber's ability for hydroxyl groups to bind to water. Each of these physicochemical properties significantly affects the analyses of fiber.

A combination of heat, chemical, and pressure treatment is an option for countries that produce mass quantities of fibrous by-products and do not have corn, soybean, and other grains for animal consumption. Types of crops grown vary by global region. Countries like Egypt and Thailand grow mass quantities of rice that produce husks or hulls as a waste product. The use of these waste products as animal feed is of interest. Such products are low in crude protein with high lignin or cellulose content, which are poorly digested by monogastric small intestine enzymes. To make these fibers practical for swine, poultry, and other non-ruminants further processing of DF is needed to improve digestibility and nutrient availability (Perez-Palencia et al., 2019). Processing, also called 'treatment' and 'purifying', changes the character of the fibrous components (Shi and Noblet, 1993; Höberg and Lindberg, 2004). A natural source is also referred to as 'raw' or 'untreated' carbohydrates. Treatment processes are used to improve the bioavailability of carbohydrates by changing the structure of polysaccharides and releasing SCFAs by depolymerizing hemicellulose and lignin. Short-chain fatty acids are known to contribute as an energy source in which countries with fibrous waste products can feed as a replacement for a corn/soy diet. Economy, climate, import-export policy, and disease prevalence are factors that affect global food distribution, leading to food scarcity in developed or underdeveloped countries. Food scarcity is a global crux that must be addressed with progressive solutions, like treating fibrous waste products for animal consumption. This chapter describes the characteristics of DF and the biological roles those characteristics play in the growth, health, and overall well-being of the weaned pig.

Weaned Pig Performance when fed Additional Dietary Fiber

Diet composition plays a role in the feed intake and intestinal development of growing pigs. The weaned pig's gastrointestinal tract undergoes significant physiological change during the transition from sow's milk to solid, dry feed, often leading to poor feed intake. Prolonged reduced feed intake causes inflammatory response upregulation, and lack of stimulation in the gut leads to poor intestinal barrier development because of shortened villi height and damaged crypt depth (Spreeuwenberg et al., 2001). Consequently, growth may be slower, morbidity and mortality may be greater; outcomes which decrease profit potential.

Previous research has shown that dietary fiber is not digestible, making diets less energy-dense, causing less energy intake and fewer gains (Chen et al., 2020; Graham et al., 1986; Shi and Noblet, 1993). However, other research on weanling pigs contrasts these findings and indicates that 2–6% fed ad libitum of fiber per kilogram of the diet does not impact the daily gains of the young pig (Pond and Mersmann, 1990; Mateos et al., 2006; Molist et al., 2009; Zhao et al., 2018). Published studies have also shown 10-30% DF in the monogastric diet can sustain growth and improve feed efficiency (Ricke et al., 1981; Anugwa et al., 1989; Senne et al., 1996; Lewton et al., 2019a). A high-fiber swine diet enlarges the gastrointestinal tract (Bohman et al., 1955; Kass et al., 1980; Pond et al., 1989), thus influencing the feed intake of the animal. Increased DF content changes the digestible energy value of a diet, leading to changes in weaned pig growth performance.

Physiologically, the growing pig digests fiber differently than a sow and should be formulated to reflect this difference. Fernandez et al. (1986) reported on average, sows digest 300 grams more crude fiber per kilogram than the young pig. In support of this finding, Le Goff

and Noblet (2001) reported that the difference came from the sow's higher rate of degradation in the hindgut. However, Lowell et al. (2015) reported an apparent total tract digestibility of NDF was greater ($P < 0.05$) for growing pigs fed wheat or canola meal compared to a gestating sow; apparent digestibility of ADF was greater ($P < 0.05$) for growing pigs fed wheat, soybean meal, or corn germ meal diets than the gestating sow. Consideration of physiological stage, net energy, and digestible amino acids should be accounted for when formulating diets to avoid diminished gains (Just, 1984). The swine industry is moving toward formulating diets on a net energy basis as it describes the true energy value of a feedstuff. This formulation is beneficial for complex diets, utilizing fibrous byproducts or low crude protein, because it accounts for the energy retained in the body. However, net energy evaluation for ingredients is expensive and time-consuming leaving opportunity for improvements with net energy formulation in the swine industry.

Dietary fiber benefits energy utilization and gastric function of the young pig by influencing the composition of microbial communities in the gut by way of SCFA production (Bai et al., 2020). A study by Chen et al. (2013) found acetate, propionate, and butyrate concentrations higher in the colon of 28 d weaned pigs fed 10% soyabean fiber; they concluded digesta resides in the colon longer where greater microbial populations are thus the higher concentration of SCFAs in the colon. Soluble dietary fibers (SDF) stimulate microbial fermentation by the production of SCFAs like acetic, propionate, and butyric acid, which contribute up to 15% of the energy maintenance requirement for a 35 - 42 kg pig (Shi and Noblet, 1993). Energy contribution and nutrient absorption is a mechanism for how SCFA improves gains, while reducing gut inflammation and regulating hormones. Jenkins et al. (2015) found that increasing insoluble dietary fiber (IDF) content in the diet (5.5 up to 51 g/kg) changed the balance between

butyrate and lactate, which improved the average daily gain of weaned pigs. Current literature has recommended a ratio of SDF to IDF at 1:7 or 1:5 to obtain benefits of growth and volatile fatty acid production (Lv et al., 2022). However, there is no confirmed SDF:IDF ratio for the growing pig. Molist et al. (2009) evaluated SCFA production on colon digesta in 15 d old, weaned pigs fed increasing contents of DF, soluble and insoluble, and reported wheat bran and sugar beet pulp-wheat bran diets increased butyric acid concentration with wheat bran pigs tending to have greater gains. Butyric acid is an energy source for colonic epithelial cell function, which contributes to colonocyte growth and proliferation for gut health maintenance. In a study conducted by Van Hees et al. (2019), creep-fed milk diets d 2-13 and dry diets d 14-25 consisting of a basal mixture, 5% pure cellulose, and 2% long-chain arabinoxylan oligosaccharide; they found acetic- and butyric acid concentrations in the mid-colon increased with the pure cellulose diet and no changes in the long-chain arabinoxylan oligosaccharide diet. This study reported that even the suckling piglet could be successful with a DF-supplemented diet, knowing that sow milk consumption has an indeterminate impact, and showed effects on large intestine fermentation and development. The production of SCFAs from a fibrous feed source contributes to energy utilization and gastric function by influencing the young pig's microbial community.

Weaned pig growth performance and intestinal morphology are directly related, and DF has been reported to improve gastric motility (Chiou et al., 1994) and digesta transit time. Jin et al. (1994) studied 10% wheat straw fed to 14 kg barrows for 14 days and determined crypt depth increased in the rectal-anal portion of the colon. Chen et al. (2013) fed 10% wheat bran fiber and determined an increase in ileal villi height: crypt depth ratio, which suggested greater intestinal integrity. The intestinal lumen barrier serves to protect the pig locally from various harmful

bacteria or irritants within the gastrointestinal tract. Fiber in the diet ranging from 2.7 - 7.9 % increased intestinal villi height and crypt depth, suggesting greater surface area development for absorption without negatively impacting growth performance (Sittiya et al., 2020). Dysfunction of the intestinal lumen causes permeable microorganisms or toxins to cross the barrier, leading to reduced growth performance and morphologic structures.

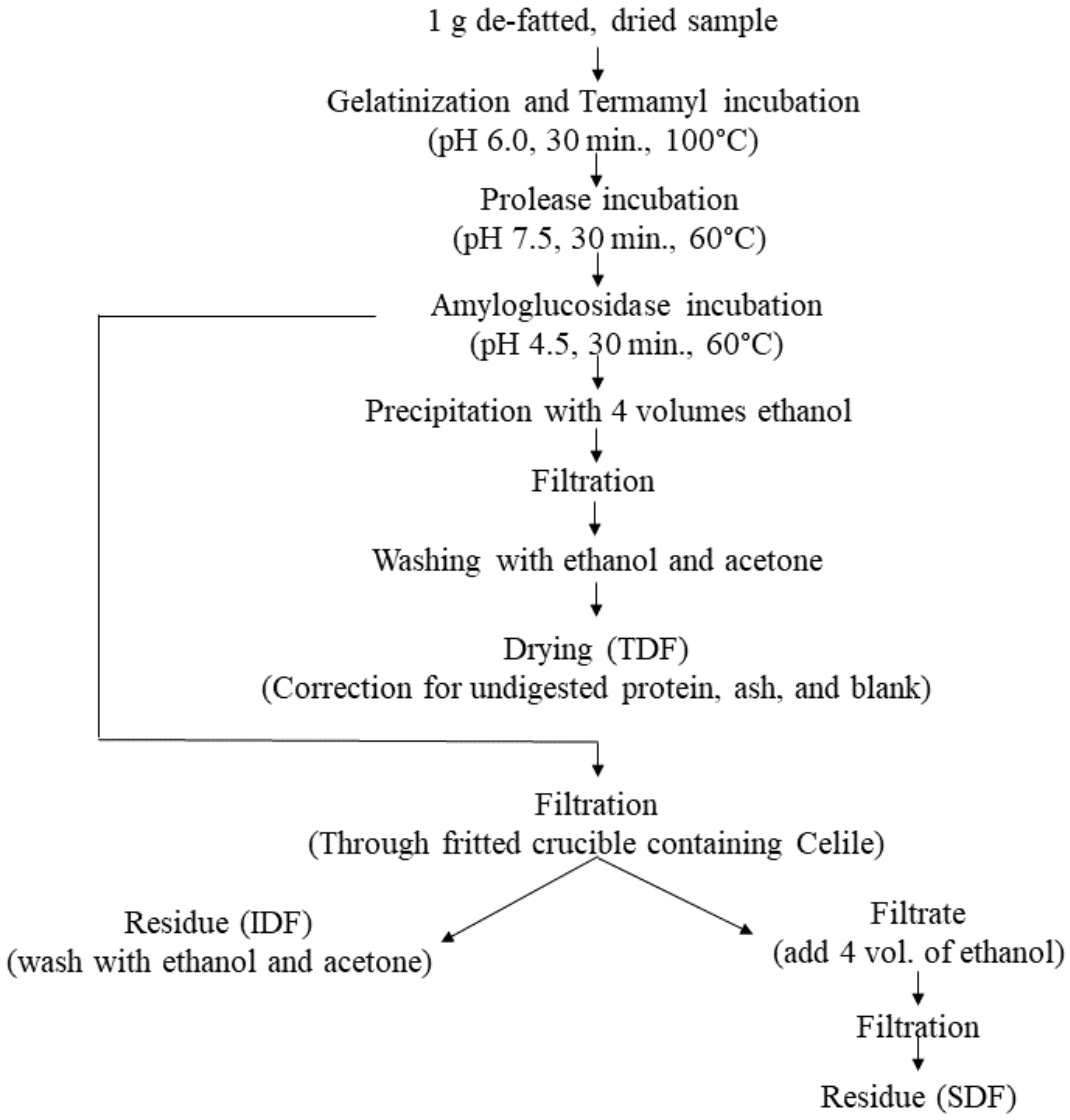
Characterization of DF

Food chemists and nutritionists have used various descriptions for DF, and the formulation with DF has evolved over time because of advances in analytical methodology. Crude fiber is the original analysis of fiber and is defined as the residue of the plant cell wall where cellulose and hemicellulose dissolve after an acid and alkali hydrolysis (Henneberg and Stohmann, 1859). This method does not acknowledge the other valuable fractions that are relevant in fiber; therefore, the need for a new analysis to incorporate the various characterizations of DF.

The term “dietary fiber” was first used by Hipsley (1953) which included the constituent’s cellulose, hemicellulose, and lignin, but Hipsley only used a grading scale rather than quantifiable numbers based on specific constituents. This started the evolution of thinking when it came to fiber characterization paired with analysis. In 1967, Van Soest and Wine developed the detergent methods known as NDF and ADF (Van Soest and Wine, 1967) to quantify fibrous constituents hemicellulose, cellulose and lignin that are considered nutritionally unavailable, insoluble residues. The detergent methods were then used to ration diets because the values could relate to bulkiness of forage and animal feed intake. To determine NDF, the whole fiber is boiled in a neutral detergent solution to extract the soluble content to leave hemicellulose, lignin, cellulose, and silicic acid. To calculate ADF, the fiber fraction is boiled in an acid detergent solution where hemicellulose is dissolved isolating cellulose, lignin, and silicic acid. In the

following work done from 1971-1976, coworkers adapted the DF term to include physiological-botanical descriptors to include digestion-resistant material from the plant cell wall (Painter and Burkitt, 1971; Burkitt et al., 1972; Trowell, 1972; Trowell, 1976). In 1976, the term "dietary fiber" was redefined as the sum of lignin, polysaccharides, and indigestible polysaccharides in the diet that are not digested by endogenous secretions of the digestive tract (Trowell, 1976). The definition was broadened because of analytic findings to include new constituents such as gums, modified cellulose, mucilage, oligosaccharide, and pectin. This new definition was widely acknowledged and led to quantifying DF portions with physiological function in analytic methods. From 1976 – 1985 efforts were put toward removing digestible from non-digestible fractions of fiber with the use of enzymes (Theander and Aman, 1978; Schweizer and Würsch, 1979; Asp and Johansson, 1981; Prosky et al., 1985). The Association of Official Analytical Chemists (AOAC) recognized the work done by these scientists and agreed to conduct a national collaborative study (Prosky et al., 1985). Officially, in 1999, the AOAC used gas chromatographic-colorimetric-gravimetric methodology to determine TDF in foods (AOAC, 1999) and was extended to measure soluble and insoluble DF together in TDF. All the AOAC fiber analytical methodologies were approved and are the results of the work done by Trowell (1972, 1976) and Prosky and others (1985).

Figure 1.1. AOAC method 32.1.17 enzymatic- gravimetric analysis of total, soluble, and insoluble dietary fiber. Adapted from (FAO, 1998)



As of today, the enzymatic-gravimetric method is used to determine TDF, SDF, and IDF (AOAC Official Method 985.29, 2005). The analytic method for TDF enzymatically hydrolyzes a dried feed sample using α-amylase and amyloglucidase to digest starches and carbohydrates. By the end of the analysis, digestible (SDF) and non-digestible (IDF) are weighed separately. As these fiber characteristics have become of interest, appropriate means for rationing and formulating are needed. There can be confusion about what fiber constituents are classified as soluble and insoluble because constituents like hemicellulose and resistant starches share soluble and insoluble properties. The solubility of hemicellulose depends on chemical structure and alkaline solution concentration; the value by the end of the analysis differs and depends on the fibrous source itself and treatment. This is also common of resistant starches that when ingested raw or untreated, will pass through the small intestine nearly intact like an IDF, and then become fermented in the large intestine acting like a soluble DF. Both hemicellulose and resistant starches are an example of why caution is used when formulating with fibrous feeds, as SDF and IDF analysis changes the relevance of overall DF.

Food chemists and nutritionists have undoubtedly broadened their knowledge of analytical methodology over time; however, we believe there is still much to learn about the values or recommendations used to formulate diets. This being based on inconsistencies between studies as analytical methods differ for experimental DF amounts.

Table 1.1. Carbohydrate characterization grouped by different analytical methods. Adapted from (Halas & Babinszky, 2014)

Carbohydrate					
N-Free Extract			Crude Fiber		
Oligosaccharide	Resistant Starch	Pectin	Hemicellulose	Cellulose	Lignin
				ADF	
				NDF	

Table 1.1. (cont'd)

Soluble Fiber	Insoluble Fiber	Lignin
Total Dietary Fiber		
Non-Starch Polysaccharide		Lignin

Soluble vs. Insoluble

It is speculated that the soluble-to-insoluble ratio in a controlled/formulated swine diet may be most insightful as these properties are responsible for systemic and local changes in the weaned pig. Soluble and insoluble DF properties work together to promote the overall health of the animal, as both contribute to fermentation but individually contribute to various behaviors like hydration, swelling, and enzymatic activity (Anderson et al., 1990; Barber et al., 2020). It is soluble and insoluble DF properties that influence metabolic health, however, there is no current recommended ratio that is consistent across the literature (Tao et al., 2019; Lv et al., 2022).

Soluble and insoluble dietary fibers have varying physiological effects which are dependent on structural and physical properties. Soluble DFs are known to dissolve in the small intestine, delay gastric emptying, and slow glucose absorption. These fibers become fermentation substrates in the hindgut for monogastric animals which then alters microbial communities and provides energy due to the utilization of SCFAs (Zhao et al., 2019; Bai et al., 2020). Common SDFs are pectin, galactomannan, and β-glucan functioning to increase the viscosity of digesta by creating a gelling agent to ease the flow and force of fluid through the gastrointestinal tract. The dissolution characteristic of SDF elicits a reduction response in glycemic and plasma cholesterol (Mudgil, 2017). Most carbohydrates leave the small intestine nearly intact and proceed to the large intestine for fermentation in the colon. Insoluble DF promotes the movement of digesta, increase fecal bulk, and bile acid secretion to aid in digestion (Mudgil, 2017). The insoluble portion of fiber has minimal nutritional value and as previously mentioned, is fiber's insoluble residues. Soluble and insoluble DF properties explain their metabolic attributes and how they

could potentially become a nutrient constraint when formulating.

Treatment of Fibrous Feedstuffs

The treatment of fibrous feedstuffs, physical and chemical, break covalent and non-covalent bonds of lignocellulolytic components reducing the plant's lignin and hemicellulose content (Bader Ul Ain et al., 2019). Thermochemical treatments modify the soluble and insoluble portions of dietary fiber (Bergner, 1981; Bader Ul Ain et al., 2019), creating a more bioavailable ingredient. In terms of nutrition, bioavailability means the portion of nutrients that are available for physiological function and ultimately utilized in systemic circulation (Fernández-García et al., 2009). In human food manufacturing, treatment processes like blanching, milling, or enzymatic have been utilized. In breakfast cereal processing, pressure-cooking treatment develops the grain's flavor and texture by transforming it to be palatable and edible through the mechanism of expanding cellularity (Caldwell et al., 2000). An experiment conducted in Australia (Srikaeo et al., 2006), mixed varieties of wheat grains at the same increasing temperatures to assess structural change. They determined that hard wheat developed a shapeless structure in comparison to soft wheat because the lack of starch content, amylose, and amylopectin in the hard wheat as heat treatment and cook time caused swelling. Swelling of wheat decreases the number of starch granules to confirm changes in the plant's raw or untreated structure. Through treatment, fiber's characteristics shift, and available nutrients change. Ultimately, the treatment process changes the physiological effect in the monogastric digestive system.

Hydrothermal, Pressure, and Chemical Treatment

Fibrous byproducts have the potential to become a main ingredient in swine diets through mechanical treatments like heat, moisture, chemicals, and pressure. These treatments improve the

digestibility of lignocellulosic biomass. One common method to accomplish this is hot water pretreatment (hydrothermal). This bioconversion uses intense heat of 200°C to lower water pH and cleave hemiacetal linkages from hemicellulose (Pérez et al., 2007). Another hydrothermal process used by Rodríguez et al. (2009) depolymerized hemicellulose at the temperature of 190°C for 15 mins. Intense heat held for a period causes the breakdown of covalent and non-covalent bonds in lignin and hemicellulose, allowing beneficial compounds like SCFAs to be available from complex fibers. Díaz et al. (2011) used a hydrothermal pre-treatment on sunflower stalks and reported that at 220°C, hemicellulose became more soluble. Another method to breakdown lignocellulosic material is pressure cooking. Weil et al. (1997) used pressure cooking as an aqueous pretreatment on corn fiber to reveal a linear correlation of increased solubility as the treatment reached a final temperature of 220°C. In recent literature, Bader Ul Ain et al. (2019) used a pressure-cooking technique to modify the soluble and insoluble portions of barley and determined pressure-cooking only slightly modified the SDF to IDF ratio. Hydrothermal and pressure-cooking treatments alone have proven to be useful in modifying fibrous plant sources (Weil et al., 1997; Rodríguez et al., 2009; Díaz et al., 2011; Bader Ul Ain et al., 2019). These methodologies have the potential to improve the utilization of fibrous ingredients for the weaned pig diet that are not commercially practiced.

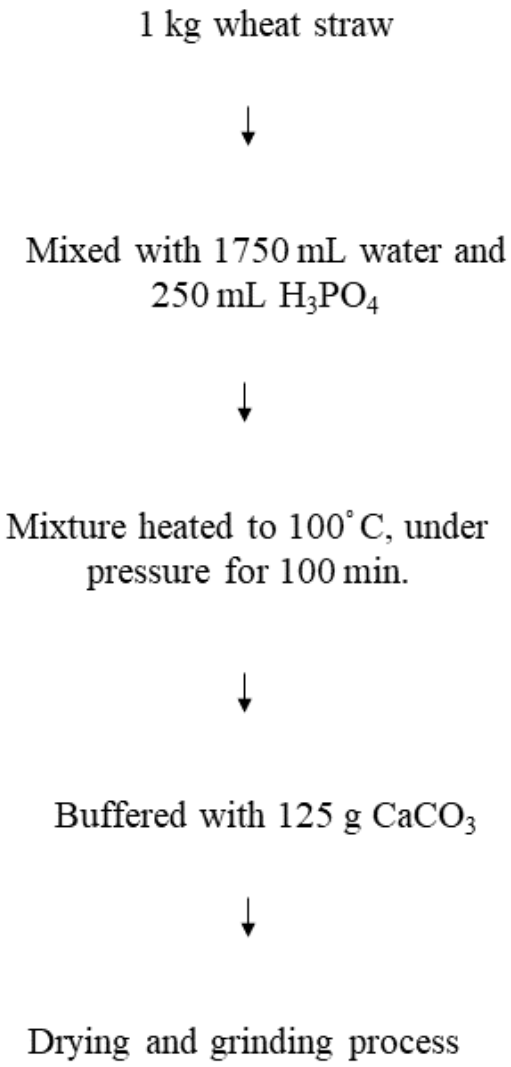
Acids or chemical treatments are an additional option for delignification of a fibrous plant source because it digests plant rigidity making the fiber a readily available ingredient. Alkali treatments have been previously reported to change cellulose and hemicellulose structure compared to other cell wall constituents, like lignin or pectin, (Rexen and Thomsen, 1976) specifically in the first 24 hr. of digestion *in vitro*. Lloyd and Wyman (2005) used a sulfuric acid concentration of 0.98% at 150°C for 5 minutes to produce a max yield of 38.3% for combined

glucose and xylan of corn stover compared to a water only treatment, producing a max yield of 56.8% of glucose and xylan at 210°C for 6 minutes. Following the work of Lloyd and Wyman, Kootstra et al. (2009a, 2009b) soaked milled wheat straw in 46 mM of maleic acid for 50 min at 170°C for a 92% glucose yield. Other nutritional profiles affected by acid treatment are crude fiber, NDF, and ADF when 0.5 N of sulfuric acid was used for a 24-hr. treatment at 25°C (Naseer et al., 2017). Chemical treatments can effectively improve the digestibility of lignocellulosic biomass, making this method another alternative to modify fibrous feeds.

The combination of heat, chemicals, and pressure can make fibrous by-products a suitable supplement in the growing pig diet because of compositional changes. Bergner (1981) indicated the use of a combination treatment did not reduce the energy intake because SCFAs were produced, and acceptable animal growth was reported. The work of Michael (2010) fed rice straw to broilers using a combination treatment and indicated normal growth. Both Bergner and Michael did not report analytic fiber values of their treated straw, however, they hypothesized that the improved gains and health of the animal was a result of the combination treatment method changing the structure and property of the straw. Lewton et al. (2019a), adapted the method from Bergner (1981) and Michael (2010), who reported analytical fiber values. In this study, all fiber composition analyses (cellulose, hemicellulose, lignin, crude fiber, NDF/ADF, TDF, and SDF/IDF) were reduced compared to the untreated wheat straw. Specifically, NDF and ADF in the untreated wheat straw were 73.99% and 48.82% respectively. After applying the combination treatment, these values were reduced to 37.39% NDF and 33.21% ADF. Other notable untreated vs treated wheat straw composition differences from the Lewton et al. (2019a) study were increased calcium, phosphorus, and ash because of calcium carbonate and phosphoric acid used during the treatment process. Calcium of the untreated wheat sample was 0.27% and

0.08% for phosphorus; when treated, these values became 4.28% and 8.29% respectively. Treatment methodologies and DF analysis vary among experiments thus the need for further elucidation if to be used for commercial swine diets.

Figure 1.2. Wheat Straw Treatment Process Used at Michigan State University. Adapted from Lewton (2019a)



Weanling Pig Health

The transition from the farrowing room into the nursery can bring immune challenges because stressors increase the young pig's susceptibility to becoming cold, starved, or ill. This is occurring at a time in which their immune system is developing. Weaning results in greater permeability of the gastrointestinal tract due to these stressors. Literature states that weaning alone causes greater secretory activity and intestinal permeability (Moeser et al., 2007). Intestinal upregulation of inflammatory cytokines is prevalent in the first two days post-weaning and correlates to a reduced feed intake (Mccracken et al., 1999; Pié et al., 2004). Nutrition intervention can indirectly mitigate the negative impact on systemic and local health of the young pig when faced with stressors (Matsumoto et al., 2023). A formulated diet of five percent pectin, a soluble fiber, was fed for four weeks ad libitum to conclude improved intestinal health and downregulated pro-inflammatory cytokine (TNF-α, IL-6) expression in the jejunum of 21 d weaned pigs (Dang et al., 2023). Pigs weaned at 21 d fed 0.5% IDF + SDF and 0.75% IDF + 0.25% SDF (first two weeks), then 0.25% IDF + 0.75% SDF (last two weeks) increased expression of ileal IL-10, an anti-inflammatory cytokine, compared to a 1% IDF diet (Chen et al., 2020). The first two weeks in the nursery is a critical time point for gut barrier establishment to improve the overall health, digestibility, and performance of the young pig where nutritional intervention can be a component in fostering that foundation.

In 2019, Lewton et al. (2019b) fed treated wheat straw to understand systemic and local health impacts; it was observed that weaned pigs fed 5 and 10% treated wheat straw improved feed efficiency and reduced gene expression of pro-inflammatory cytokines TNF-α ($P < 0.05$) and ant-inflammatory cytokine IL-10 ($P < 0.05$) in the ascending colon. In the following study from Michigan State University (2020), 5% treated wheat straw reduced pro-inflammatory cytokines

IL-6 and IL-12 ($P < 0.05$) using the same treatment method as mentioned previously. The impact of treated DF on systemic and local immune function using immunological biomarkers of the weaned pig is not fully understood.

The young pig's immune system is frequently challenged during the transition through production phases and continues to be active until the time of harvest; thus, the gut must be set up for success to fight off prevalent nursery stressors and diseases. Immunological tools can be used to assess changes in systemic and local health, such as quantitative measures on components of the immune system. Components such as immunoglobulins, proteins, and cytokines work in conjunction to stabilize the immune system in response to stressors (Dinarello, 2000; Späth, 2000; Zimmerman and Veerkamp, 2002). These components help define what is happening to the pig during stressful events and the overall health implications that are exhibited physically. As we learn about the mechanisms to improve gut barrier function, nutritionists can pair systemic and local tools to understand how DF's metabolic attributes play a role in the digestive system.

Immunological Biomarkers

Immunological biomarkers are quantitative measures that objectively determine if illness or biological abnormalities are present (Llibre and Duffy, 2018). Immune biomarkers can be white blood cells, immunoglobulins, cytokines, and proteins. Biomarker assessment used in conjunction with clinical outcome assessments is a way to predict the outcome of health when disease/illness is apparent (Burke and Henson, 1993; Burke, 2004).

Systemic Markers: Immunoglobulins are heterodimeric proteins that bind to invading pathogens which neutralize and destroy any foreign molecule. B-cells, a type of white blood cell formed in bone marrow, secrete immunoglobulins, and are presented in five classes:

immunoglobulin A (IgA), G (IgG), M (IgM), D (IgD), and E (IgE). These classes are based on physiochemical, structure, and immunological properties (Späth, 2000). Systemic biomarker IgA is predominantly found on mucosal surfaces, like the gastrointestinal tract, playing an important role as the first line of defense from mucosal pathogens. Immunoglobulin A exhibits protective functions by interacting with specific receptors and immune mediators to provide insight on health status. Blood collection for IgA assessment can be done using serum or plasma. Serum IgA refers to IgA found in the blood stream and prevalent in immune defense, they are closely associated with immune response and conditions involving mucosal surfaces. Plasma IgA represents mucosal protection, immunological tolerance of the mucosa, and systemic immunity (Kerr, 1990), whereas plasma IgA suggests broader health conditions. Both serum and plasma IgA should be read in conjunction with current exhibited clinical signs, other medical tests, and medical history. Immunoglobulin A antibodies protect mucosal surfaces and are secreted into the intestinal lumen to prevent attachment of harmful antigens. Another systemic biomarker that can be used are fatty acid-binding proteins (FABP). These binding proteins are tissue-specific, where lipid-binding proteins transport fatty acids between cellular membranes (Zimmerman and Veerkamp, 2002). Fatty acid binding proteins are intracellular proteins that coordinate lipid trafficking and the transportation of long-chain fatty acids to provide an energy source to cells. Tissues can express more than one type of fatty acid binding protein at a time, however intestinal fatty acid binding proteins (iFABP) are only found in enterocytes which are epithelial cells in the small intestine. After injury, iFABPs are released quickly into circulation and considered for intestinal integrity assessment. Immunoglobulins and fatty acid-binding proteins are useful in determining circulatory response because of their response to intestinal integrity.

Local Markers: Cytokines are proteins produced rapidly in response to inflammation,

infection, or trauma to regulate the innate and adaptive immune system through immune cells by acting as a chemical messenger (IQWiG, 2006). Innate immunity, also called general or non-specific immunity, responds to germs or foreign substances with the purpose of containing infection locally. If the innate immune system does not eliminate the presence or influence of the antigen, then adaptive immunity identifies and targets the specific infectious agent. An unknown or new substance in the system takes longer for recognition, but once identified the immune system remembers it for a quicker response the next time, this being immunity.

Cytokine characterizations such as pro and anti-inflammatory have intertwined functions and work to balance the immune reactions during stressors or infection. Pro-inflammatory cytokines are stimulated during immune challenges, specifically during the innate immune (Kaiser, 2022) response to recruit and activate immune cells, acute phase proteins, and induce fever as the challenge advances. Pro-inflammatory cytokines modulate the immune system by coordinating cell-mediated immune signals to the infected area promoting upregulation and signal healing. Anti-inflammatory cytokines downregulate the pro-inflammatory cytokines and are immunomodulators that resolve inflammatory cascades to initiate tissue repair (Dinarello, 2000). A feedback mechanism engages to control immune cells from becoming overactive and eventually prevents chronic inflammation. Cytokine classification is universally accepted into five classifications: interleukins, interferons, colony-stimulating factors, tumor necrosis factors, and growth factors. Interleukins are synthesized upon infectious stimuli, homeostasis, tissue repair, or stress response. They are secreted from immune cells, and some non-immune cells, as a signaling molecule to regulate cell-mediated immunity. Once secreted, the proteins bind to specific receptors to change gene transcription and protein expression through signaling cascades (Foster, 2001). Cytokines can be used for tissue-specific responses in which a cytokine pattern

reveals tissue damage, inflammation, or autoimmune diseases.

Pro-Inflammatory Cytokines: The presence of pro-inflammatory cytokines causes clinical signs of illness (fever, inflammation, or tissue apoptosis) and is useful in determining health status. Interleukin 6 (IL-6) stimulates the production of acute-phase proteins, which mirrors the intensity of inflammation. Interleukin 6 is also responsible for generating cellular immune responses to an inflamed site. The IL-6 cytokine induces an immune response by destroying the localized injured area within a short period of time during the acute phase; prolonging inflammation dictates the transition from acute to chronic inflammation (Gabay, 2006). Interleukin 12 (IL-12) regulates T helper cells, type 1 and 2, to facilitate cell immunity against microbial pathogens. The main initiator of immune cells is T helper cells which function to detect and fight infection. Interleukin 12 is activated by inflammatory cells and is the link from innate resistance to adaptive immunity (Gately et al., 1998). Tumor necrosis factor was originally discovered and named for causing cell death of tumor cells but recently has been recognized for inflammation, immunity, and tissue homeostasis functions. Immunometabolism is a term that came along with the discovery of tumor necrosis factor, being an adipokine to interpret metabolic reprogramming contributing to homeostasis of healthy or diseased individuals (Sethi and Hotamisligil, 2021). Specifically, tumor necrosis factor alpha (TNF-α) is activated by macrophages to induce signal transduction pathways for cell proliferation, differentiation, and survival. As an early immune mediator, TNF-α localizes inflammation and plays a role in the transition to chronic inflammation. Overproduction of TNF- α is harmful especially when it escapes the localized area to enter systemic circulation; dysfunction of TNF- α alters physiological events and leads to autoimmune diseases or in some cases, death (Strieter et al., 1993). Pro-inflammatory cytokines can be used as a tool to assess the health of an animal. To

best identify the reason for illness, clinical assessments should be paired with quantitative measures of these cytokines.

Anti-Inflammatory Cytokine: Interleukin 10 (IL-10) inhibits T cell and macrophage activation, primarily to cease inflammatory responses through transmembrane receptor complexes. During infection, IL-10 reduces tissue damage by limiting the immune response to pathogens and inhibiting antigen presentation and pro-inflammatory cytokine expression (Sabat et al., 2010). Interleukin 10 also regulates the growth and differentiation of B cells, natural killer cells, and T helper cells depending on the stimulation present in affected tissue. Many local immune cells express IL-10 but regulate differently per cell. Regardless of source, well-timed production of IL-10 is beneficial to the host by clearing infection while minimizing damage.

Immunological biomarkers are useful tools to understand what is happening in the live animal to predict a clinical outcome. As immunoglobulins, FABPs, and cytokines play critical roles in immune health, they are determinates of stress or illness if abnormalities are present in the systemic and local immune system. These focused biomarkers allow the reader to understand the potential health benefits systemic, and local immune responses of young pigs fed DF.

Conclusion

The inclusion of dietary fiber is important in feed as the industry transitions away from antibiotic use. Nutritional intervention for weaned pigs can be used to reduce morbidity, mortality, and resist enteric and other disease challenges by improving intestinal integrity (Xiong et al., 2019). It is important to understand what DF characteristics impact physiological health while noting changes in nutrient composition between DF sources. Literature has reported DF in the growing pig can be comparable to, if not enhance, growth performance parameters, morphology, and immune markers of pigs fed standard commercial diets (Bikker et al., 2006;

Hedemann et al., 2006; Weber et al., 2008; Liu et al., 2018). However, some refute the impact of DF on the young pig diet because of the potential to reduce energy density, growth rate, and feed intake. However, feeding DF is dependent on the physiological stage and sufficient net energy to achieve optimal performance.

Further investigation of DF on the impact of immune health is needed with the use of immunological biomarkers and treatment. Biomarkers used as quantitative tools paired with clinical tests present a clear picture of DF on the gastrointestinal tract health and how a weanling pig utilizes fiber as a feedstuff. Further utilization of DF as an ingredient can be accomplished when subjecting DF to treatment with hydrothermal, chemical, pressure, or a combination of these methods to make the plant source more bioavailable. The changing of DF structure and therefore analytic composition are associated with systemic and local development of the young pig's intestinal tract (Lewton et al., 2019b; Chen et al., 2020; Matsumoto et al., 2023).

In Chapter 2, the effect of treated wheat straw in the diet was investigated. Immune health parameters were used as assessment tools to determine if the weaned pig can maintain health when a vaccine-induced immune challenge is present. As previously mentioned, the first two weeks in the nursery are critical for gut development in which DF can positively foster that foundation through systemic and local mechanisms (Chen et al., 2020; Dang et al., 2023; Matsumoto et al., 2023). Given that DF has down-regulated pro-inflammatory cytokine expression, we hypothesized that an immune-challenged, weaned pig fed treated wheat straw would experience an improved gut health response.

In summary, the work highlighted here aims to explain if the weaned pig can consume a fibrous diet, without antimicrobials or antibiotics, and grow acceptably while sustaining health. There is a gap in understanding which fiber characteristics are important, and how to modify

fiber to reflect those characteristics to improve nutrient utilization and health of weanling pigs fed DF.

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CHAPTER 2: UNDERSTANDING THE HEALTH BENEFITS OF FEEDING TREATED WHEAT STRAW TO WEANLING PIGS

Abstract

An experiment was conducted to evaluate the impact of 5% dietary treated wheat straw on the local and systemic health of nursery pigs experiencing a vaccination-induced immune challenge. Twenty-four barrows (PIC 800 x Yorkshire) were weaned at 27.7 ± 0.8 d (8.6 ± 1.2 kg) and randomly assigned to four cohorts based on weight and parentage, with six pigs per cohort. Cohorts were randomly assigned to two treatments: a control treatment (CON), which was a commercially alike standard dietary nursery program and a treated wheat straw (TWS) treatment, which was a dietary program like the CON treatment but included 5% treated wheat straw. After being fed experimental diets for 21 d, pigs were challenged with two vaccinations, the injectable Ingelvac® CircoFLEX™ Porcine Circovirus Type 2 vaccine, and an orally administered Enterisol® *Lawsonia intracellularis* vaccine. All pigs were sacrificed on d 42. Blood samples were collected to assess plasma IgA and iFABP concentration. Mucosal scrapings from segments of the distal ileum and ascending colon were removed for the analysis of localized immunological markers IL-6, IL-10, IL-12, and TNF-α. Serum PCV2 titers and mucosal *L. intracellularis* titers confirmed an active immune response to vaccination. The ascending colon of TWS treatment increased crypt depth ($P = 0.05$) and muscle thickness ($P < 0.01$). Gene expression of TWS treatment for colonic TNF-α and IL-6 ($P < 0.05$) were greater. Overall, nursery diets with 5% treated wheat straw impacted the health of vaccine-challenged pigs, as seen with changes in colonic morphology and the expression of immune markers. The weanling pig's response to 5% treated wheat straw in the diet depends on the health of the pig, leaving the practice of using dietary fiber to alleviate illness associated with weaning of the pig unestablished as a recommendation across all farms.

Introduction

Demand for and production of livestock increases as the world's population continues to increase. By 2050, farming and livestock production is expected to double, with 70% of land dedicated to agriculture (FAO, 2006). It is essential to find livestock feed alternatives that are non-competitive with human-grade ingredients. By utilizing byproducts like wheat straw or corn stalks, livestock termed "up-cyclers" can confront food security and environmental challenges (Ominski et al., 2021). Fibrous byproducts are potential alternative feed sources, like straws, dried distillers' grains, or grain milling coproducts. For swine, further processing of byproducts is needed to improve digestibility and nutrient availability (Perez-Palencia et al., 2019).

There is evidence that the treatment process makes straw a suitable supplement in the weaned pig diet. Bergner (1981) reported that growing pigs fed 10% hydrolyzed straw meal improved the health, growth, and efficiency of pig production. Lewton et al. (2019) treated wheat straw with acid, heat, and pressure (adapted from methods of Bergner, 1981 and Michael, 2010) and fed 5 and 10% treated wheat straw to weanling pigs. It was determined that growth performance can be maintained up to 10% treated wheat straw and suggested improved immune function in the colon of those fed dietary fiber (DF). These treatments may also contribute to enhanced immune function in the colon, partially attributed to DF.

Processing fibrous ingredients can significantly change the structure and analytical composition, resulting in improved digestibility (Rodríguez et al., 2009; Díaz et al., 2011). Therefore, the present experiment aims to explore the relationship between DF, its impact on intestinal health, and its influence on the immune system. A relationship that recent studies have shed light on. Previous literature examining the effects of DF and inflammatory cytokine pathways found an increase in IL-6 and IL-10 of ileal tissue was observed for weanling pigs fed

7.5% soluble dried distillers' grain (Weber et al., 2008). The roles of these cytokines in the immune system are complex, and the effects of DF on IL-6 targeting pathway and inflammatory response are not completely understood. Furthermore, Liu et al. (2018) found that feeding 5% corn bran to weaned pigs had no effect on pro-inflammatory cytokine TNF-α and increased anti-inflammatory cytokine IL-10. Hence there is need for further research on the systemic health of weaned pigs fed DF to advance the understanding of promoting well-being.

Fiber research has highlighted local health benefits in the weaned pig diet revealing changes in the small intestine. The inclusion of 19% barley hulls in the diet increased villi length and enhanced mucosal enzyme activity, altering gastric motility and nutrient absorption capacity (Hedemann et al., 2006; Chiou et al., 1994; Sittiya et al., 2020). Longer villi increase the surface area and nutrient absorption, therefore improving the diffusion of digestible nutrients commonly seen in fibrous feedstuffs. These findings emphasize the role DF can play in enhancing intestinal health.

The prospect of DF enhancing health led to the objective of this experiment, which is to understand the systemic and local health benefits of feeding 5% treated wheat straw to a vaccine-induced immune-challenged weaned pig. The hypothesis was that an immune-challenged, weaned pig-fed treated wheat straw would experience an improved gut health response with the provision of supplemental DF.

Materials and Methods

Animals, Experimental Design, and Housing

The experiment protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University (PROTO202200151). This study was conducted between September and October 2022 at the Michigan State Swine Teaching and

Research Center. Dams had been vaccinated pre-breeding for erysipelas, leptospirosis, and parvovirus. Newborn piglets were processed in one to two days: ear notching, tail docking, and 1.0 mL of iron dextran (200 mg/mL). Between 7 and 10 d, pigs were castrated and received an additional 1.0 mL iron dextran injection. Twenty-four barrows (PIC 800 × Yorkshire) were weaned at 28 ± 0.8 d (8.6 ± 1.2 kg) of age. This study was a randomized block design based on weight and parentage to provide two light cohorts and two heavy cohorts, with one of each assigned to either dietary treatment. Cohorts were randomly assigned to pens in an environmentally controlled nursery room, with six pigs per pen. An additional pig was included in each cohort as a backup source of samples. None were needed. All pens were 1.22 x 1.83 m, with tri-bar flooring, vertical stainless steel rod gates, stainless steel single-sided two-space (each 20.3 x 20.3 cm) feeders (SD Industries, LLC Alexandria, SD), and one nipple drinker. The space per pig was confirmed using the formula elucidated by Gonyou et al. (2006): $k * bw^{.667}$ = area per pig (k = 0.0347). At the end of the nursery phase, pigs were projected to weigh about 34 kg and require a maximum of 0.3646 m^2 per pig. Pens (1.22 x 1.83 m) provided 2.23 m^2. The feeder occupied 0.23 m^2 of that space, providing 2.00 m^2 of actual pen space for pigs. Thus, this value was rounded up to arrive at six pigs per pen (2.00 m^2 ÷ 0.365 m^2 = 5.5 pigs per pen). Rooms were operated on an all-in/all-out system and disinfected using bleaching (15.6 mL/L) 2-5 days prior to pig placement.

Dietary Treatments and Feeding

Two dietary inclusion concentrations (0 or 5%) of treated wheat straw were used: a **control diet (CON)** with 0% treated wheat straw and diets formulated like the CON diets but containing **5% treated wheat straw (TWS).** The study was a three-phase dietary, commercially alike program (Table 2.1). The three nursery phases were d 0-10, 11-21, and 22-42, with d 0

representing weaning (27.7 ± 0.75 d). All diets were formulated with commercial nursery nutritional complexity, with dietary copper and zinc at or slightly above the suggested levels of the NRC (2012). No antimicrobials were included in the feed or drinking water. All diets were mixed at Michigan State University Swine Farm using a 113-kg paddle ribbon mixer. Micro ingredients were pre-mixed at the Animal Science Nutrition Laboratory (Michigan State University, Department of Animal Science, East Lansing, MI, USA), before mixing with macro ingredients. Feed and water were provided ad libitum.

Wheat Straw Treatment and Preparation: This study followed a patented method (Michael, 2010) for treating wheat straw. Treatment involved, per kg of wheat straw, soaked straw in 2000 mL of distilled water and 250 mL of O-Phosphoric acid, 85% (Fisher Scientific, Hampton, NH, USA) for approximately five mins with each addition. In the bottom of a 15 L pressure cooker (Buffalo Cookware, Kuala Lumpur, MY) 1750 mL of distilled water was added prior to the soaked wheat straw addition. Following soaking, the mixture was placed in the pressure cooker and tightly sealed, heated to 100°C under pressure for 100 mins with a cooling phase of 20 mins. Once cooled, 125 g of calcium carbonate was slowly mixed in as a solution buffer. After thoroughly mixing, cooked wheat straw was placed in aluminum drying pans and an initial weight was recorded. The mixture was dried in a drying oven (Fisher Scientific, Hampton, NH, USA) set to 70°C, and wheat straw was confirmed dry using a weight comparison system for an average of 24-30 hr. total dry time. The dried straw was ground finely through a one mm screen using a Wiley mill micro grinder (Thomas Scientific, Swedesboro, NJ, USA) and stored in 1.81 kg brown paper bags at the Animal Science Nutrition Laboratory (Michigan State University, Department of Animal Science, East Lansing, MI, USA) at room temperature until mixing.

Analysis of Feed and Treated Straw: Chemical analyses were performed for each dietary phase along with samples of ground wheat straw and treated wheat straw (Table 2.2). Composite samples taken from individual feeders per treatment and phase were shipped to the University of Missouri Agricultural Experimental Station Chemical Laboratory (Columbia, MO, USA) for analysis following the standard methods of the AOAC (2006). Two additional samples were sent of ground untreated and treated wheat straw for eight samples analyzed (Table 2.3). The following analyses were performed: neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose, lignin, hemicellulose, total dietary fiber (TDF), soluble dietary fiber (SDF), insoluble dietary fiber (IDF), individual amino acids, and proximate analysis (crude protein, crude fiber, crude fat, moisture, and ash). Neutral detergent fiber was determined from AOAC 56, 1352-1356, 1973; acid detergent fiber was determined according to AOAC official method 973.18 (A-D, 2006). Lignin was determined using sulfuric acid (AOAC Official Method 973.18 (A-D), 2006), and hemicellulose was determined as the difference between NDF and ADF. Total dietary fiber was determined by way of the enzymatic gravimetric method (AOAC Official Method 985.29, 2006). Individual amino acids were determined according to the Kjeldahl method, with tryptophan calculated separately via ion exchange chromatography (AOAC Official Method 982.30 E, 2006). According to the Experiment Station Chemical Laboratory adapted protocols, nitrogen was analyzed using the copper catalyst Kjeldahl method, and crude protein was calculated from nitrogen using 6.25 as a conversion factor (AOAC Official Method 984.13 (A-D), 2006). Proximate analyses included crude fiber via the fritted glass crucible method and crude fat via ether extraction (AOAC Official Method 978.10, 920.39 (A), 2006). Ash and remaining moisture were analyzed by vacuum oven (AOAC 942.05, 934.01, 2006).

Data Recording and Sample Collection

Pig weight and pen feed consumption were monitored weekly. The average daily gain (ADG) was collected by weighing each individual pig and averaging weights within the pen. Each pen's feeder was vacuumed out and the remaining feed was subtracted from the total additions of the week to estimate average daily feed intake (ADFI). Feed efficiency (G:F) was calculated by dividing ADG by its corresponding ADFI. These growth measures were not intended to be statistically compared as growth performance was not the objective of this study.

Three weeks into the study (d 21), every pig received a one mL dose intramuscular injection of Ingelvac® CircoFLEX™ Porcine Circovirus Type 2 (PCV2), and an oral inoculation two mL dose of Enterisol® *Lawsonia intracellularis* (Boehringer Ingelheim, Ingelheim, DE). On d 28, post-vaccination, 12 pigs, six per treatment, were randomly selected for blood serum samples, and on d 35 the other 12 pigs, six per treatment, not sampled previously, were sampled for titer determination. Serum was collected via jugular venipuncture in five mL vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, USA). On d 42, all euthanized pigs, 10 per treatment, were sampled for final titer determination of serum and intestinal tissue were submitted to Iowa State University Veterinary Diagnostic Lab (Ames, IA, USA) for analysis following their protocols.

On d 42, prior to euthanasia, 10 pigs from each cohort were selected at random for sedation using Telazol-T (2.50 mg/kg), Ketamine-K (1.25 mg/kg), and Xylazine-X (1.25 mg/kg); used as TKX (0.025 mL/kg) injectable given intramuscular (gluteus medius) with a 22 G needle. A final blood sample was taken during sedation for plasma IgA and iFABP concentration analyses. Blood plasma was collected via jugular venipuncture in 10 mL EDTA vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, USA). Once confirmed sedated, pigs were euthanized via sodium phenobarbital (Euthasol; 1mL/4.50 kg) overdose intra-cardiac single injection with 18 G

needles. Absence of respiration, heartbeat, and corneal reflex used for confirmation of death, then distal ileum and proximal colon excised. The ileum and colon were sectioned using a string cut along the peritoneum, approximately 152 mm. Every pig's thymus and spleen were located after intestinal segments and weighed.

Upon removal, each intestinal segment was opened lengthwise and placed in chilled 1× PBS. Once cleaned, a small portion of each sample was collected for histology. Histology tissue samples were prepared by placing the tissue in appropriately labeled plastic cassettes, mucosa side up, and immediately fixed in Carnoy's 2000 (FXCAR2GAL, StatLab, McKinney, TX) for 24 hr. Mucosa from the ileum and ascending colon were collected by mucosal scrapes on a metal pan over ice. Two glass slides were used to gently remove the mucosa from the muscular layer of the tissue. Mucosa was immediately flash frozen with liquid nitrogen, placed in whirl pack bags, rolled up, and placed in a container with liquid nitrogen. Samples were then moved to -80°C for long-term storage.

Serum and Plasma Analyses

Serum and plasma blood tubes were placed immediately on ice and centrifuged for 20 min at 3,000 rpm. Serum and plasma were separated and aliquoted into appropriately labeled centrifuge tubes. Plasma samples were stored at -80°C until IgA and iFABP analysis. Immunoglobulin A was analyzed using a porcine-specific IgA ELISA kit (E101-102, Bethyl Laboratories, Inc., Montgomery, TX, USA). Samples and materials were thawed for two hr. prior to dilution and followed the kit protocol except for washing the plate 2× prior to loading samples. The dilution scheme for the IgA kit was 1:25,000 samples to diluent. The standard curve was established by a standard serial dilution scheme (1.37-1,000 ng/ml). Optical density set absorbance of 450 nm within 30 min of adding the stop solution. Intestinal fatty acid binding protein was analyzed

using a human FABP2/I-FABP immunoassay (DFBP20, R&D Systems, Inc, Minneapolis, MN, USA) which had a porcine sensitivity. Samples were thawed for two hr. prior to dilution (25 µL of sample + 300 µL of diluent) and followed the kit protocol. The standard curve was established by a standard serial dilution scheme (0-1,000 ng/ml) and an optical density set absorbance of 450 nm within 30 min of adding the stop solution.

Mucosal Sample Analyses

Mucosal scrapes obtained from the distal ileum and proximal colon were immediately flash-frozen in liquid nitrogen.

Gene Expression: Total RNA was isolated from 30 mg of mucosal scrapes using the RNeasy Mini kit (74106, Qiagen, Hilden, DE) and RNase-Free DNase Set (79254, Qiagen, Hilden, DE). The first strand of cDNA was synthesized from 480 ng of RNA using Thermo Scientific Maxima First Strand cDNA Synthesis Kit for RT-qPCR with dsDNase (K1671, Thermo-Fisher, Waltham, MA, USA). Real-time qPCR was used to determine the relative quantities of transcripts of the specific genes (IL-6, 10, 12, and TNF-α). All PCR reactions used Power SYBR Green (4367659, Applied Biosystems, Waltham, MA, USA) and the QuantStudio™ 3 system (Applied Biosystems, Waltham, MA, USA) and were subjected to a melt curve analysis to validate the absence of nonspecific products. Data presented as 2-ΔΔCT in gene expression relative to the CON group, first normalized to the reference gene RPL4 (Jonge et al., 2007; Kim et al., 2023).

Protein Abundance Assay: Total protein was isolated from mucosal samples using Mammalian Protein Extraction Reagent (MPER, PI78501, Thermo-Fisher, Waltham, MA, USA) with protease inhibitor cocktail (11697498001, Roche, Basel, Switzerland) and phosphatase inhibitor cocktail (PI78426, Thermo-Fisher, Waltham, MA, USA), and protein was quantified using Pierce BCA Protein Assay Kit (PI23227, Thermo-Fisher, Waltham, MA, USA). The

expression of specific proteins was analyzed via ELISA commercial kits and followed the kit protocols. Interleukin-6 was analyzed using a porcine-specific IL-6 ELISA kit (ELP-IL6, RayBiotech, Inc., Norcross, GA, USA) and diluted two-fold. Interleukin-10 and TNF-α were analyzed using porcine-specific kits (P1000 and PTA00, R&D Systems, Inc., Minneapolis, MN, USA), both diluted 2-fold. Interleukin-12 was analyzed by ELISA kit (ESIL 12A, Invitrogen, Carlsbad, CA, USA) loaded undiluted because PCR data showed low enough values to compare to the literature. Standard curves were established by standard serial dilution scheme: IL-6 (0-10,000 pg/ml), IL-10 (0-2,000 pg/ml), TNF-α (0-1,500 pg/ml), and IL-12 (0-1,000 ng/ml). Each kit was read at 450 nm within 30 min of adding the stop solution.

Histopathological Analyses

Ileal and ascending colon tissue samples for histology were immediately fixed in Carnoy's 2000 (FXCAR2GAL, StatLab, McKinney, TX). Samples were transferred to 70% ethanol post 24 hr. post collection. Samples were then taken to Michigan State University Investigative Histopathology Laboratory (East Lansing, MI, USA) for hematoxylin and eosin staining. According to their procedures, tissue samples previously fixed in 10% Neutral Buffered Formalin were processed and vacuum infiltrated with paraffin on the Sakura VIP 2000 tissue processor and embedded by the ThermoFisher HistoCentre III embedding station. Excess paraffin was removed from the edges of cooled samples onto a Reichert Jung 2030 rotary microtome exposing tissue samples. Once cooled, the sample was delicately sectioned at four to five microns. After sectioning, slides were dried at a held temperature of 56°C in an incubator for 2-24 hr. for optimal adhesion. Finally, slides were stained with routine hematoxylin and eosin methods, followed by adding a cover slip with synthetic mounting media to ensure indefinite preservation and optical viewing. Slides viewed using a Leica DM750 brightfield microscope in

20× magnification for goblet cell number and 10× magnification for villi height and crypt depth (Leica LAS EZ software, Buffalo Grove, IL, USA). Ileal samples measured villi height and crypt depth, selecting a minimum of 15 representative samples of villi and crypt. Ascending colon samples measured villi height, crypt depth, goblet cell concentration, and muscle thickness. Excluding goblet cell concentration, a minimum of 15 representatives are used for measurements of villi, crypt, and muscle thickness. Total goblet cells per crypt and μm of crypt depth evaluated using 15 representative crypts. Photos taken for analysis of villi and crypt were also used for goblet cell evaluation.

Statistical Analysis

An animal was the experimental unit, following a plan for 10 observations per treatment group at the time of d 42 sample collection. Plasma and mucosal data were analyzed using an unpaired t-test in GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). Morphology, titer, and organ weight data were analyzed using the PROC GLM procedure and the pairwise comparison of LSMeans in SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA). Body weight at d 42 was included as a covariate in the PROC GLM models when significant at $P < 0.10$. Performance data was analyzed using SAS PROC GLIMMIX procedure (SAS Institute, Inc., Cary, NC,USA) with pen as experimental unit and the weight block included as a covariate in the model. Treatment differences were considered significant at $P \leq 0.05$ and tendencies at $P > 0.05$ and ≤ 0.10.

Results and Discussion

General Comments – Diets, Pig Growth

All pigs experienced commercially acceptable growth rates and remained on trial for the entire six-week period. None of the animals on study were treated. Average daily gain, average

daily feed intake, and feed efficiency are shown in Table 2.4. This experiment used only four pens, a heavy and light weight pen with each treatment. Average daily gain ($P = 0.002$) and G:F ($P = 0.001$) in the third week were improved for the TWS pigs. Pigs on the TWS treatment consumed less feed in the fifth ($P = 0.03$) and sixth ($P = 0.01$) week than the CON treatment pigs. These differences did not contribute to overall differences in these performance measures. It is notable that the L. *intracellularis* challenge mounted the greatest response in the sixth week of the study (Figure 2.1).

Diets in this study were formulated to be isonitrogenous and isocaloric. However, upon analysis, there was a difference of 0.28% in lysine between the CON and TWS diets in Phase 1 (Table 2.2). Despite this difference, both diets exceeded the NRC (2012) requirement of 1.34% for the 28-d weaned pig. However, the excess lysine may have led to differences in lysine's relationship to other essential amino acids. Methionine is recommended to be formulated at 28% of 100% lysine, and based on a lysine content of 1.82%, methionine should be 0.51%. According to the analysis (Table 2.2), the CON diet methionine content was 0.45%, which might potentially hinder growth. The analyzed amino acid content differed from the formulated constraints and could be attributed to errors in weighing, mixing, and feed sampling. Given that the lysine content of both diets surpassed the NRC (2012) requirement, impacts on growth or health, although unknown, are believed to be minimal if any.

PCV2 and L. intracellularis

Serum PCV2 titer confirmed that the vaccine challenge was effective in causing an active immune response in pigs over three weeks (Figure 2.1). According to Iowa State University VDL, on d 7 after vaccination, all pigs were negative for PCV2 titers. On d 14 post-vaccination, there was one suspect and, one positive for the TWS treatment and two positives for the CON

treatment for PCV2 titer. On d 21 post-vaccination, all samples were positive except two negatives for the TWS treatment and two negatives for the CON treatment for the PCV2 titer. This study did not compare PCV2 titers between dietary treatments as the Iowa State University VDL results provided were not appropriate for quantitative comparison. The laboratory results were reported as S/P and titer. Titer is calculated as: titer = 53 (e3.2x), where x is S/P of sample. Where S/P is the ratio of the sample's optical density to the positive control serum optical density. This titer result confirmed the presence of an antibody titer and that infection had or had not occurred. Previous studies to evaluate a circulatory titer response to DF under a PCV2 vaccine challenge have not been found in the literature.

Serum L. *intracellularis* titers were negative for both treatments 7 and 14 d after vaccination, and 21 d there were two positives in the TWS group and two suspects in the CON group. As with circulatory PCV2 titers, the Iowa State University VDL *L. intracellularis* results were not appropriate for quantitative treatment comparison.

All ileal and colonic mucosal samples obtained 21 d post-vaccination, were positive for *L. intracellularis*, confirming that all pigs had responded at a gut level to that vaccination. The titer data reported as CT allowed for quantitative comparison. The immune response tended to differ ($P = 0.08$) in the ileum and differed in the colon ($P = 0.02$) with dietary treatment, indicating greater antibodies produced by TWS pigs than CON pigs (Table 2.5). This measurement demonstrates that the TWS pigs responded differently than CON pigs. The reason why this difference occurred is unclear. However, per the ISU VDL, L. *intracellularis* qPCR assay targets the 16S ribosomal RNA gene. Without the differentiation between vaccine or wild-type strains, there is potential *L.intracellularis* was prevalent on farm as an endemic. This is the only known study to report DF impact on mucosal response to the *L. intracellularis* attenuated live vaccine.

The titer results for both intestinal locations confirmed that the pigs were experiencing an immune response. *L. intracellularis* is a species of bacteria that causes proliferative enteropathy leading to lethargy, anorexia, diarrhea, and an increase in mortality, with early symptoms being in the intestine (Hansen et al., 2022). None of these clinical indications of disease were observed in the current study.

Organ Weight

Spleen and thymus weights were recorded on d 42 post euthanasia because of their involvement in immune function. The weights of the spleen ($P = 0.17$) and thymus ($P = 0.90$) did not differ between dietary treatments (Table 2.6). Increases in visceral organ weights, like kidney, liver, or pancreas, with the addition of dietary fibrous by-products have been reported previously (Mersmann et al., 1987; Pond and Mersmann, 1990; Agyekum and Nyachoti, 2017). There are no known published studies comparing measures of spleen or thymus weight for weaned pigs fed a dietary fiber treatment challenged with *L. intracellularis* or PCV2. Moreover, in literature there is conflicting results whether the presence of enteric disease impacts immune organ weight (Bayyari, 1997; Balan, 2011; Manafi, 2016; Abdullah, 2020).

Intestinal Morphology

Ileal morphology was not different between treatments (Table 2.7). Moore et al. (1988) fed 15-20% high fiber diets (oat hulls, soybean hulls, and alfalfa meal) to young pigs (9.7 kg initial BW) and determined ileal villi were tongue and leaf-like which appeared similar to the control-basal diet-fed pigs. This contrasts with other literature (Chen et al., 2013) that reported diets with 10-12% insoluble fiber content increased ileal villi height suggesting pigs consuming greater amounts of fiber might be healthier because of changes in the gut structure.

In the ascending colon, 5% treated wheat straw pigs had greater crypt depth ($P = 0.05$) than control pigs suggesting enhanced gut motility and surface area to improve what absorption occurs in the colon. This agrees with Jin et al. (1994), where crypt depth in the rectal-anal portion of the colon was increased in pigs fed 10% wheat straw. Colonic muscle thickness was greater in TWS pigs ($P < 0.01$). This increase could be from insoluble dietary fiber, which is known to increase feed bulk, stimulate muscular contractions, and muscle thickness (Chiou et al., 1994). This study's colonic morphology results disagree with work previously conducted within the Michigan State University research group (Lewton et al., 2019), who observed no differences in muscle thickness or crypt depth.

Dietary fiber has shown notable effects on enteric health, particularly in terms of resistance to bacterial infections like *E.coli* (Smith and Halls, 1968; Bertschinger et al., 1978). This protective effect is particularly associated with insoluble dietary fiber which may enhance the pig's ability to oppose infection by increasing mucosal secretion (Smith and Halls, 1968; Zebrowska et al., 1983). Enhancements in enteric health may additionally be attributed to the reduction in digesta transit time observed by Kass et al., 1980, which subsequently reduces available substrate for pathogens. This study sought to explore the relationship between dietary fiber and intestinal morphology. While crypt depth can be influenced by factors such as cell proliferation, there were no significant differences in goblet cell count per villi ($P = 0.55$) within the ascending colon (Table 2.7). These results agree with those of Piel et al. (2007) observed no changes in goblet cell number in weaned pigs fed 12.1-21.8 % DM of total fiber with up to 15% insoluble fiber. Similarly, Vila et al. (2018) noted no change in the expression of goblet cell numbers between dietary treatments (a corn and soybean meal, 40% corn-dried distillers' grain solubles, or 40% wheat middlings). However, Paturi et al. (2018) found that goblet cell

concentration increased in the colon of rats fed 7.5% fiber diets (cellulose/pectin, apple fiber, and broccoli fiber). While morphologic findings add valuable insight, these measures alone are not a direct indicator of illness but serve as an observation of digestive function, overall health, and the well-being of the animal.

The composition of DF can vary significantly, with the soluble and insoluble components playing unique roles in influencing intestinal function. The ratio of soluble to insoluble has been studied little. Lv et al. (2022) suggested a ratio of 1:5 or 1:7 of SDF to IDF for weaned pigs, although this recommendation remains unexplored. This study's experimental diets do not align with Lv et al. (2022) study's suggested ratio, thus this study cannot report a ratio based on these findings. Notably, the TWS experimental diet increased TDF and IDF. It is possible that IDF could be responsible for the observed increase in colonic crypt depth and muscle thickness (Chiou et al., 1994; Jin et al., 1994).

The wheat straw samples showed no difference in SDF when subjected to treatment (Table 2.3), a pattern consistent with SDF in the experimental diets, except in the first phase. It is crucial to recognize the nutritional value of the treated vs. untreated wheat straw sample, as it can change by processing. In this experiment, the treatment process accomplished structural and nutritional change in the wheat straw (Table 2.3), leading to an increase in decrease TDF. In agreement, Dharmaraj and Malleshi (2010) reported that finger millet's total DF decreased after decortication. The native source was 17.10 g/100g compared to 10.10 g/100g after decortication due to removal of the seed coat. The treatment process in this chapter causes swelling and separation of the seed coat, comparable to the study previously mentioned. Processing techniques, like thermal and chemical, have been reported to change the raw source proximate and TDF composition (Beloshapka et al., 2016). In light of this understanding, it is evident that

morphologic changes observed were attributed to the modification of wheat straw composition following treatment.

Immunological Markers

Immunological markers are biological molecules that can be used to quantitatively measure immune system activity, dysfunction, or response. This work focused on local and circulatory markers to assess the response to dietary treatment, infection, and inflammation in terms of gene and protein expression.

Circulatory Markers: Immunoglobulin A protein abundance did not differ between dietary treatments ($P = 0.33$). It is known that IgA works to protect the body from infection while building immune tolerance, suggesting that the immune challenge called for a greater IgA response that TWS could not overcome. The results from the current study differ from those reported (Matsumoto et al., 2023; Zhang et al., 2023), where plasma IgA increased in pigs fed 3% SDF. Pigs in that study had not received an immunologic challenge. In this study, the IgA measured 1.21mg/mL (Table 2.8), which was greater than the 0.468 mg/mL and 0.482 mg/mL measured by Lewton et al. (2019) and Polniak et al. (2023). The immune challenge in the present study led to greater circulatory IgA, possibly too great an increase for the TWS treatment to enhance further.

Intestinal fatty acid binding protein did not differ in blood plasma ($P = 0.83$). Intestinal fatty acid binding protein is responsible for the binding and transporting of dietary fatty acids from the epithelial layer of the intestines into the bloodstream. Elevated expression of iFABP indicates intestinal disorders or damage, and because this study did not have elevated iFABP it is thought that treated wheat straw was not damaging or compromising the intestinal lining.

Local Markers: Gene expression of immunological markers did not differ between the two dietary treatments (Table 2.8). In the ileum, TNF-α, IL-6, IL-10, and IL-12 did not differ between CON or TWS treatment ($P > 0.05$). In the ascending colon, IL-10 and IL-12 showed no significance between dietary treatment ($P > 0.05$). This agrees with work done by Vila et al. (2018), who fed 40% soluble dried distiller grain and 30% wheat middlings of the total diet; they observed no changes in IL-6, IL-10, and TNF-α in ileal cytokine expression, although the pigs were not challenged. However, the results of Li et al. (2019) disagree; their research showed that the ileal gene expression of TNF-α tended to increase with the feeding of 10% soluble fiber diets under an immune challenge.

In the ascending colon mucosa, dietary-treated wheat straw increased the gene expression of TNF-α ($P < 0.01$) and IL-6 ($P = 0.03$). An increase in the pro-inflammatory cytokine IL-6 in the colon of the weaned pig with the feeding of dietary fiber has been reported previously by Pie et al. (2007). However, these findings contradict the work of Lewton (2019) who reported no change in TNF-α and a decrease in IL-6 expression. The mechanisms for why two pro-inflammatory cytokines increased in the ascending colon are unknown, as DF effects of lessening expression of these markers have been seen previously (Sweeney et al., 2012; Zeitz et al., 2019; Liebl et al., 2022). The *L. intracellularis* vaccine-challenge could be the reason TNF-α and IL-6 were expressed greater, because they are responsible for healing processes when disease is present(Leite et al., 2019).

Protein abundance in ileal or colonic mucosa did not differ between dietary treatments (Table 2.9). In the ileum, TNF-α, IL-6, IL-10, and IL-12 did not differ between treatments ($P > 0.05$). Same was true in the ascending colon for TNF-α, IL-6, IL-10, and IL-12 ($P > 0.05$). This disagreed with Nogueira et al. (2013), who reported that oral inoculation of *L. intracellularis*

expressed higher mucosal protein abundance of TNF-α and IL-6 by d 17 of the experiment. Gene and protein expression can differ if there is a discrepancy between transcription and translation processes that can be caused by multiple mechanisms, one such being a regulatory mechanism. Although not analyzed in this study, it is possible in the colon, treated wheat straw influenced short-chain fatty acid (SCFA) production, which plays a role in regulating gene expression. Interleukin-6 is classically known as a pro-inflammatory cytokine but has the potential to become anti-inflammatory when bound to membrane-bound receptor IL-6 receptor α known as classic signaling. In this study, it is possible that gene expression of IL-6 was an anti-inflammatory response caused by the feeding of fiber that increased SCFA production. It has been reported that SCFAs can regulate intestinal macrophages to influence signaling pathways, through histone deacetylase or G protein-coupled receptors, to perform classic signaling (Smith et al., 2013, Chang et al., 2014; Garbers and Rose-John, 2018).

In this study, greater colonic *L.intracellularis* titer count in combination with an increase in gene expression of pro-inflammatory cytokines and differences in morphology, all in the ascending colon, were observed. This combination is congruent and consistent with previous reports of DF effects in the large intestine of swine. These findings suggest that DF increased the inflammatory response in the ascending colon. It is unclear if the inflammatory indices suggest these changes are detrimental or beneficial to health, both are plausible. If the addition of treated wheat straw was detrimental, expected increase in iFABP or villi damage would be observed, neither were observed in this study. However, the series of experiments at Michigan State University suggest the inclusion of treated wheat straw has shown to support normal growth and enhanced nutrient utilization, suggesting this source is beneficial to pig health. The observation of differences in *L.intracellularis* gene and antibody expression only in the ascending colon is

puzzling, as *L.intracellularis* historically presents in the ileum. These results suggest that in an immune challenged condition, the animal could not utilize the treated wheat straw diet to further enhance gut health.

Conclusions

Vaccine challenged pigs fed treated wheat straw elicited a complex local and systemic response compared to control pigs in the experiment. Treated wheat straw did not appear to augment the immune response occurring in the small intestine during a time when the small intestine was rapidly developing its immunity to *L. intracellularis*. In this study, it was observed that feeding treated wheat straw increased pro-inflammatory cytokine production in the ascending colon. A result, which is inconsistent with the results of previous studies, some using and others not using an immune challenge experimental design. There was no change in anti-inflammatory marker expression in the colon when feeding DF, also disagreeing with other studies. The change in colonic morphology indicates better gut health. The health benefit of feeding DF depends on the health of the pig. A standardized practice of including dietary fiber in all nursery nutrition programs is not confirmed by these results. Although statistical differences were observed, a further experiment with greater replication and power is necessary to draw final conclusions about treatment effects on pig growth. Future directions should assess growth performance and feed efficiency from weaning to harvest. If and how much DF prevents illness or enhances restoration in illness in the weaned pig remains intriguingly unknown.

TABLES

Table 2.1. Dietary components across diets, within each phase, as-fed basis

	Phase 1 diets (d0-7)		**Phase 2 diets (d7-14)**		**Phase 3 diets (d14-28)**	
Ingredient %	**CON**[1]	**TWS**	**CON**	**TWS**	**CON**	**TWS**
Corn grain	44.7	39.2	48.2	42.7	53.5	48.6
Soybean meal, 47%	17.2	18.1	24.0	24.9	28.6	28.8
Corn DDGS, 12% oil	5.0	5.0	7.5	7.5	10.0	10.0
Dried whey 72% Lactose	20.0	20.0	10.0	10.0	-	-
Fish meal	3.0	3.0	3.0	3.0	-	-
Spray dried bovine plasma	4.0	4.0	-	-	-	-
Corn oil	0.50	0.55	0.50	0.55	1.0	1.02
Dextrose	0.94	2.26	2.35	3.67	2.45	3.74
Calcium carbonate, 38.5% Ca	1.01	1.38	0.83	1.19	0.95	1.32
Mono calcium phosphate, 21.5% P	2.08	0.01	2.05	-	2.05	-
Salt	0.3	0.3	0.35	0.35	0.4	0.4
VTM premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine HCL	0.33	0.31	0.44	0.42	0.45	0.45
DL-Methionine	0.18	0.18	0.15	0.15	0.16	0.16
L-Threonine	0.12	0.11	0.16	0.16	0.16	0.16
L-Tryptophan	0.03	0.02	0.04	0.04	0.03	0.03
L-Valine	0.05	0.04	0.08	0.07	0.04	0.05
Citric acid	0.30	0.30	0.15	0.15	-	-
Treated wheat straw	-	5.0	-	5.0	-	5.0
Total	**100**	**100**	**100**	**100**	**100**	**100**

[1] CON = basal control ; TWS = basal control with 5% treated wheat straw

[2]VTM premix provided the following vitamins and microminerals in the following concentrations: Zinc 83.4 g/kg, iron 66.7 g/kg, manganese 33.4 g/kg, copper 10 g/kg, iodine 0.3 g/kg, selenium 0.2 g/kg, vitamin A 7,363 KIU, vitamin D 1,177 KIU, vitamin E 44,112 IU, menadione 1.5 g, vitamin B12 0.02 g, riboflavin 4.7 g, pantothenic acid 14.7 g, niacin 29.4 g, thiamine 0.7 g, pyridoxine 2.9 g, folic acid 1.1 g, and biotin 0.1 g

Table 2.2. Analyzed composition of experimental diets, as-fed basis

	Phase 1 (d 0-10)		Phase 2 (d 11-21)		Phase 3 (d 22-42)	
Item, %	**CON**[1]	**TWS**	**CON**	**TWS**	**CON**	**TWS**
GE, calories/100 g	348	346	342	339	342	338
Crude protein	21.38	22.38	23.48	20.11	22.39	21.89
Crude fat	4.82	4.93	4.43	4.56	4.26	4.46
Lys	1.82	1.54	1.56	1.5	1.5	1.42
Met	0.45	0.43	0.55	0.44	0.48	0.47
Thr	1.04	0.98	0.98	0.92	0.95	0.89
Crude fiber	1.86	3.31	2.38	3.88	2.72	4.16
NDF[2]	7.63	9.12	8.85	10.39	9.30	11.31
ADF[2]	3.92	5.56	4.53	6.42	5.64	7.29
Hemicellulose	3.71	3.56	4.32	3.98	3.67	4.02
Cellulose	3.44	4.82	4.01	5.18	4.68	6.19
Lignin	0.91	1.19	0.91	1.50	1.30	1.52
Total dietary fiber	9.79	13.33	10.80	13.89	12.72	14.01
SDF[3]	0.21	0.13	0.19	0.20	0.22	0.21
IDF[3]	9.44	13.22	10.69	13.67	11.98	13.72

[1]CON = basal control; TWS = 5% treated wheat straw

[2]NDF = neutral detergent fiber; ADF = acid detergent fiber

[3]SDF = soluble dietary fiber; IDF = insoluble dietary fiber

Analyses conducted at the University of Missouri Agricultural Experimental Station Chemical Laboratory (Columbia, MO, USA)

Table 2.3. Analyzed composition of wheat straw, both untreated and treated

Item, %	**Untreated straw**	**Treated straw**
Moisture	8.32	6.97
Gross Energy, calories/100 g	175	166
Crude protein	2.67	1.40
Crude fat	0.74	4.53
Ash	6.69	30.3
Calcium	0.17	3.55
Phosphorus	0.04	8.34
Crude fiber	42.27	26.81
NDF[1]	75.31	39.88
ADF[1]	53.65	37.26
Hemi-cellulose	21.66	2.62
Cellulose	42.08	28.79
Lignin	8.68	7.06
Total dietary fiber	80.07	42.63
SDF[2]	< 0.08	< 0.08
IDF[2]	78.96	40.91

[1]NDF = neutral detergent fiber; ADF = acid detergent fiber

[2]SDF = soluble dietary fiber; IDF = insoluble dietary fiber

Analyses conducted at University of Missouri Agricultural Experimental Station Chemical Laboratory (Columbia, MO, USA)

Table 2.4. Weekly growth performance of pigs fed control and treated wheat straw (TWS) diets[1]

Item	Control	TWS	SEM	*P*-value
ADG, kg				
Wk 1	0.22	0.18	0.03	0.40
Wk 2	0.50	0.45	0.03	0.21
Wk 3	0.41[a]	0.57[b]	0.03	0.002
Wk 4	0.75	0.69	0.03	0.22
Wk 5	0.88	0.81	0.03	0.12
Wk 6	0.87	0.84	0.03	0.51
Overall	0.60	0.59	0.01	0.36
ADFI, kg				
Wk 1	0.33	0.34	0.04	0.85
Wk 2	0.60	0.58	0.04	0.68
Wk 3	0.67	0.72	0.04	0.36
Wk 4	1.14	1.10	0.04	0.52
Wk 5	1.37[a]	1.23[b]	0.04	0.03
Wk 6	1.45[a]	1.28[b]	0.04	0.01
Overall	0.93	0.87	0.01	0.11
G:F				
Wk 1	0.67[a]	0.54[b]	0.03	0.01
Wk 2	0.84	0.78	0.03	0.16
Wk 3	0.60[a]	0.79[b]	0.03	0.001
Wk 4	0.65	0.63	0.03	0.49
Wk 5	0.64	0.66	0.03	0.63
Wk 6	0.61	0.66	0.03	0.22
Overall	0.67	0.68	0.01	0.73

[1] Performance data taken from n = 2 pens per treatment, 1 pen per weight block (light and heavy), 6 pigs per pen

[a,b]Values in a common row lacking a common superscript differ ($P \leq 0.05$)

Table 2.5. Titers of *L. intracellularis* in the mucosa of ileum and ascending colon (CT)

Item[a]	**CON**[1]	**TWS**	***P* – value**
Ileum[b]	19.1 ± 1.23	16.0 ± 1.16	0.08
Ascending colon[c]	24.1 ± 1.29	19.5 ± 1.29	0.02

[1]CON = basal control; TWS = basal control with 5% treated wheat straw

[a] LSMean ± standard error of the mean

[b] For ileal titers, n=9 and 10 for CON and TWS, respectively

[c] For ascending colon n=10 for both treatments

Table 2.6. Effects of dietary treatment on spleen and thymus weight

Items[a]	**CON**[1]	**TWS**	***P* - value**
Spleen, g	152 ± 9.6	172 ± 9.6	0.17
Thymus, g	41.9 ± 3.5	41.3 ± 3.5	0.90

[1]CON = basal control; TWS = basal control with 5% treated wheat straw

[a] LSMean ± standard error of the mean. For all items included in this table, n=10 observations per treatment. d42 body weight included in the analysis as a covariate

Table 2.7. Effects of control (CON) and 5% treated wheat straw (TWS) on the morphology of the distal ileum and the proximal colon of the young pig

Item[a]	CON	TWS	*P* - value
Ileum[b]			
Mucosal villi height, µm	0.273 ± 0.011	0.288 ± 0.011	0.36
Peyer's patch villi height, µm	0.230 ± 0.010	0.242 ± 0.010	0.40
Mucosal crypt depth, µm	0.462 ± 0.017	0.491 ± 0.016	0.23
Peyer's patch crypt depth, µm	0.471 ± 0.015	0.473 ± 0.015	0.88
Ascending colon[b]			
Villi height, µm	0.396 ± 0.011	0.384 ± 0.011	0.42
Crypt depth, µm	0.067 ± 0.008	0.092 ± 0.008	0.05
Muscle thickness, µm	0.319 ± 0.017	0.392 ± 0.017	< 0.01
Goblet cell count	30.4 ± 2.180	28.5 ± 2.18	0.55

[a] LSMean ± standard error of the mean. For all items included in this table n=10 observations per treatment. d42 body weight included in the statistical analysis as a covariate

[b] For mucosal villi height and mucosal crypt depth, n=9 and 10 for CON and TWS, respectively for both. For all other items presented in this table, n=10 for both treatments

Table 2.8. Gene expression of intestinal mucosa immunological markers of pigs 5% treated wheat straw (TWS) relative to control (CON)

Item[a]	**CON**	**TWS**	**SEDM**[b]	***P* - value**
Ileum[c]				
TNF-α	1.0	0.78	0.182	0.25
IL-6	1.0	1.37	0.343	0.30
IL-10	1.0	1.51	0.365	0.18
IL-12	1.0	2.55	1.58	0.34
Ascending colon[d]				
TNF-α	1.0	2.03	0.285	< 0.01
IL-6	1.0	1.70	0.302	0.03
IL-10	1.0	1.74	0.488	0.15
IL-12	1.0	1.71	0.651	0.29

[a] Mean

[b] Standard error of the difference between means using an unpaired t-test

[c] For ileal items n=10 and 9 for CON and TWS, respectively

[d] For, ascending colon IL-10 and IL-12 n=10 and 9 for CON and TWS, respectively. For ascending colon TNF-α and IL-6, n=10 for both treatments

Table 2.9. Effect of dietary treatment on immunological markers in both blood plasma and intestinal mucosa protein abundance

Item[a]	CON[1]	TWS	SEDM[b]	*P* - value
Plasma[c]				
IgA, mg/mL	1.2	1.4	2.4	0.33
IFABP, pg/mL	355	361	26.5	0.83
Ileum (pg/mL)[d]				
TNF-α	125	121	28.6	0.88
IL-6	43	47	7.8	0.62
IL-10	247	346	95.5	0.32
IL-12	361	330	48.5	0.53
Ascending colon (pg/mL)[e]				
TNF-α	129	146	22.4	0.44
IL-6	47	49	6.1	0.74
IL-10	226	173	36.2	0.16
IL-12	305	267	38.6	0.34

[1]CON = basal control; TWS = basal control with 5% treated wheat straw

[a] Mean

[b] Standard error of the difference between means using an unpaired t-test

[c] For plasma IgA, n=9 and 10 for CON and TWS, respectively, and for iFABP, n=10 for both treatments

[d] For ileal IL-6 n=10 for both treatments. For ileal TNF-α and IL-12, n=10 and 9 for CON and TWS, respectively. For ileal IL-10, n=9 and 8 for CON and TWS, respectively

[e] For colonic IL-6, n=9 and 10 for CON and TWS, respectively. For other colonic markers n=9 for both treatments

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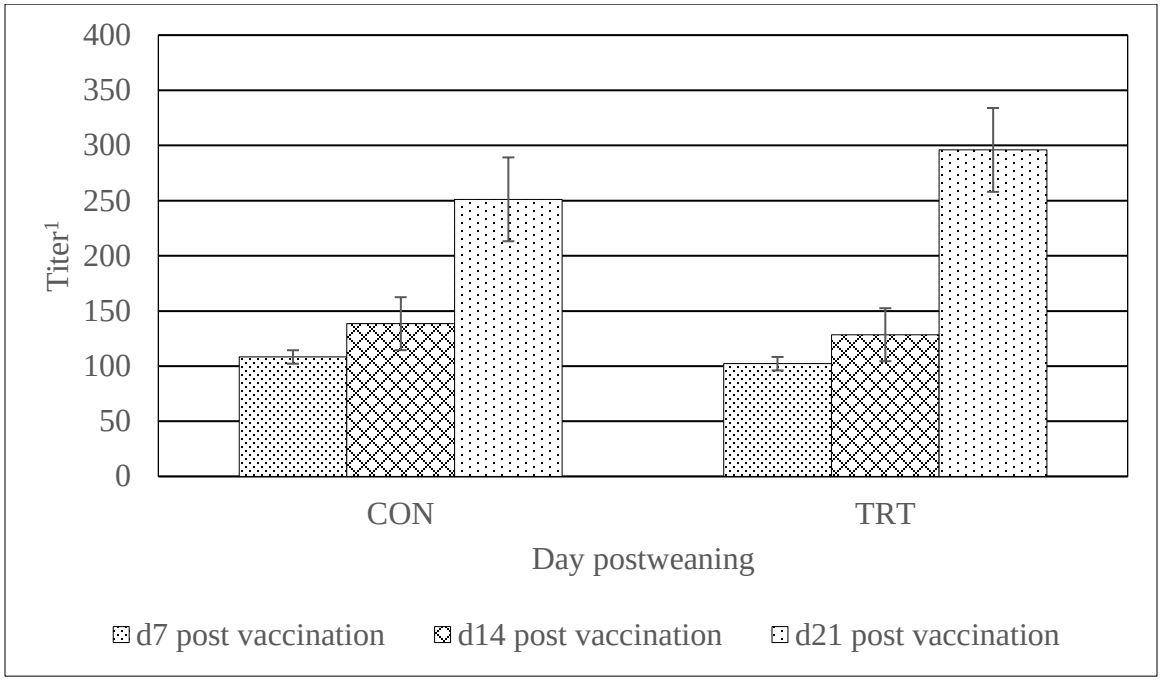
APPENDIX

Table 2.10. Gene Primer Sequences

Gene	Primer	5’ to 3’ Sequence
TNF-α	Forward	GGGGTCCTTGGGTTTGGATT
	Reverse	TTGGAACCCAAGCTTCCCTG
IL-6	Forward	AAAGAATCCAGACAAAGCCACC
	Reverse	TCCACTCGTTCTGTGACTGCA
IL-10	Forward	GAGCCAACTGCACTTCCA
	Reverse	TCAGGACAAATAGCCCACTAGCTT
IL-12	Forward	CCCTGAAGAAGACGGCATCA
	Reverse	ATGGTCAGGGTTTTGCCAGT
RPL4	Forward	GGCGTAAAGCTGCTACCCTC
	Reverse	GGATCTCTGGGCTTTTCAAGATT

Figure 2.1. Serum PCV2 titers (ISU VDL) on d 7, 14, and 21 after PCV2 vaccination



[1]Titer is calculated from the S/P. Titer = 53 ($e^{3.2x}$), where x is S/P of sample

On d 7 and 14, the "Result" for all samples, as reported by the ISU VDL were "Neg"

On d21, the "Result" for 8 of 10 samples per treatment as reported by the ISU VDL were "Pos". There were two samples per treatment reported as "Neg." on d21