Potential prebiotic effect of canola meal non-starch polysaccharide hydrolysis products in weaned piglets challenged with enterotoxigenic *Escherichia coli* F4

By

Xiaoxiao Zhang

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
In partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Animal Science
University of Manitoba
Winnipeg

ABSTRACT

Weaning period is a challenging time for piglets because piglets meet many different stressors from environment and changed diet. Weaned piglets are vulnerable to postweaning diarrhea, which could lead to economic losses in the pig industry. Antibiotic growth promoters (AGPs) have been used in the pig industry for many years in order to improve growth and decrease the diarrhea of weaned piglets. However, many countries have banned and limited the use of AGPs, finding replacement to AGPs is essential. Researchers found that enzyme hydrolysis products (HP) derived from non-starch polysaccharide (NSP) may be helpful to balance the gut health in weaned piglets. Thus, our experiment was to study the effect of NSP HP extracted from diets as the alternatives to AGPs on weaned pigs. In this study, responses of weaned piglets to an oral challenge with enterotoxigenic *Escherichia coli* F4 (ETEC) when fed diets containing canola meal (CM) HP were investigated. The HP were obtained by incubating CM with a multi-carbohydrase (MC) blend. After challenging with ETEC F4, weaned piglets fed with HP-containing diets had higher G: F than those piglets fed without any feed additives when challenged with ETEC. Piglets fed diets containing HP showed lower jejunum pH and fecal scores than piglets fed the control diet. For gene expressions, the HP group showed a lower level of *TNF-a*, *IL-6*, toll like receptor 2 (*TLR2*) as well as toll like receptor 4 (*TLR4*) compared with non-feed additive group. Feeding HP resulted in higher *BCOAF* and *lactobacillus* in the colon digesta of piglets. In conclusion, piglets fed diets with CM NSP HP exhibited less severe ETEC-enteritis and CM NSP HP could be alternatives to AGPs.

ACKNOWLEDGEMENTS

First, I would like to show my appreciation to my supervisor and mentor, Dr. Martin Nyachoti for giving me a chance to do this project, believing in me and supporting my study through meetings and comments. His enthusiastic attitude and enthusiasm for animal nutrition inspired me to continue my PhD studies. As well, I appreciate Dr. Anna Rogiewicz, who took on the role of my committee member, for her support, suggestions, comments and encouragement, which greatly benefited me during my master program. I thank Dr. Chengbo Yang, my committee member, for his patience, suggestions, and encouragement and for taking time to review my thesis.

This project was supported by funding from Canola Council of Canada. I also acknowledge the financial support that I received from the University of Manitoba, with this support I did my research successfully and complete my master's degree.

Many thanks to my friend Bonjin Koo for guidance, encouragement, and many suggestions. As well, the support and encouragement of my colleagues and friends indeed helped a lot during my last years in Winnipeg. These include Atanas Karamanov, Dr. Anna Rogiewicz, Debora Muratori Holanda, Jichen Song, Jinyoung Lee and Olumide Adeshakin. Special thanks to Lan Shi and Fernando Esposito, for their assistance in my lab analysis.

Thank you to my family members for all their support in China during last two years. My two amazing parents who always supported my choices and encouraged me to study more and live boldly. My special appreciation is for my brother and sister-in-law who are working in animal science. They encouraged me to go further study in our field. In the end, my deepest thanks and love are extended to my grandfather for his love and support. He made me realize what I really like when I was very young.

DECICATION

My dear parents Huanyong and Fengling

My brother and my sister-in-law (Rui, Junhua)

My Grandfather Haifeng

FOREWORD

Part of this thesis was presented as a poster at the online meeting of ASAS-CSAS in July 2021. The reference format is this thesis is in APA format. This thesis was prepared following the format of manuscript prepared during my master's degree program in animal science.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

GIT	Gastrointestinal tract
PWC	Postweaning colibacillosis
ETEC	Enterotoxigenic *Escherichia coli*
AGPs	Antimicrobial growth promoters
FDA	Food and drug administration
NSP	Non-starch polysaccharides
CE	Carbohydrase enzymes
PWD	Postweaning diarrhea
LAB	Lactic acid bacteria
FOS	Fructooligosaccharides
GOS	Galactooligosaccharides
SCFA	Short chain fatty acids
O_2	Oxygen
CO_2	Carbon dioxide
DM	Dry matter
TEER	Transepithelial electrical resistance
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADG	Average daily gain
ADFI	Average daily feed intake
VH	Villus height
CD	Crypt depth
BCFA	Branched chain volatile fatty acids
CBP	Canola bioactive peptides

1.0 GENERAL INTRODUCTION

During the weaning process, piglets experience a serious nutritional stress because the piglet's diet of milk from sows is suddenly shifted to a solid diet. Weaned piglets have poor gut function and are susceptible to postweaning diarrhea, weaning anorexia and malnutrition, all of which are connected with gastrointestinal tract (GIT) disturbances. At weaning, various changes in gastrointestinal physiology happen in piglets, therapy reducing the capacity of digestion and absorption capacity and enteric diseases. Changes in the weaning process are critical factors in postweaning colibacillosis (PWC) as a result of the hemolytic enterotoxigenic *Escherichia coli* (ETEC). As well, PWC may lead an economic loss in the pig industry. This is attributable to the fact that all factors involved in weaning provide favorable circumstances for E. coli multiplication in the small intestine (Fairbrother et al., 2005). As a result, appropriate replacement or strategies to enhance the growth performance and GIT ecology of weaned piglets should be further explored, and ETEC-challenged model were commonly utilized for assessing solutions for ETEC-induced diarrhea in the pig industry.

Antimicrobial growth promoters (AGPs) have been utilised as the effective feed additives in the pig industry for a long time to protect weaned piglets against pathogenic bacteria and improve health pf pigs. However, long-term use of AGPs in the pig industry, on the other hand, has been linked to the possibility of bactericidal antibiotic resistance Thus, the use of AGPs was limited and banned in many countries As a result, greater research into alternate or replacement methods for boosting growth performance and preserving intestinal health in piglets is required.

Recently, scientists found that dietary components might be used as a non-antibiotic alternative to AGPs. Our earlier research demonstrated that the enzyme hydrolysis products (HP) derived from soybean meal (SBM) or canola meal (CM) that had already been digested by enzyme increased the net fluid and solute absorption of ETEC-infected pig segments (Kiarie et al., 2010). Due to the low cost and high nutritional content in CM, it has been commonly used in swine diets. Canola meal, on the other hand, includes a high concentration of NSP, which have been labelled "anti-nutritive factor" according to the detrimental effect on energy and nutrient use. This is because CM NSP are very insoluble in water and hence cannot be digested by pigs due to their lack of

endogenous NSP digestion enzymes. The primary non-cellulosic, pectic polysaccharides and aromatic polymer lignin found in canola cell walls interface with the cellulose, generating a hard structure and reinforcing the cell wall of plant (Knudsen, 2001). As a consequence, the cell walls of canola can protect against chemical breakdown and physical damage.

Carbohydrase enzymes (CE) have been studied and utilized previously to degrade NSP in CM and has been shown to release the energy and nutrients from the cell wall of NSP (Kiarie et al., 2008). This newly created multi-enzyme carbohydrate preparation has been demonstrated to depolymerize up to 35% of CM NSP (Radfar et al., 2016). Hydrolysis products (HP) such as simple sugars, oligosaccharides as well as low molecular weight polysaccharides are formed during the digestion of CM NSP HP. Many xylo-oligomers, mano-oligomers, and gluco-oligomers may act as prebiotics. There is some evidence that these polysaccharides, or NSP HP, can assist in the gastrointestinal development by promoting the growth of beneficial *Lactobacillus* spp. in the gut and providing binding sites for pathogenic bacteria, such as Salmonella, thereby reducing attachment and colonization (Jia et al., 2009). Other experiments demonstrated that NSP HP of CM may protect against ETEC infection in weaned piglet intestinal segments (Kiarie et al., 2008). This is because the NSP HP cannot be absorbed by the host but will be accessible as substrates for microbial fermentation in the gut, hence changing their composition. Carbohydrase enzymes (CE) can digest the NSP and galacto-oligomers, gluco-oligomers, manno-oligomers, or xylo-oligomers may be formed during the depolymerization of CM NSP. Such oligomers have the prebiotic effect of facilitating those beneficial bacteria such like *Bifidobacterium* and *Lactobacillus spp*., thereby inhibiting the proliferation of pathogenic bacteria species in the GIT of weaned piglets (Radfar et al., 2016). Additionally, CE degrade NSP, releasing a variety of HP in the process. These NSP HP may help develop a healthy and balanced intestinal microbiota, and their carbohydrates operate as comparable attachment sites for pathogenic bacteria to prebiotics (Jia et al., 2009). Thus, NSP HP may help in the creation of a healthy gut by acting as a substrate for bacteria and modulating their composition.

However, whether CM NSP HP have beneficial effects on enhancing the gut health, immune system and increasing the growth performance in piglets remains to be further determined. Thus, the main objectives of the research were followings:

1. to investigate the effect of CM NSP HP on growth performance, gut health and immune system of weaned piglets challenged with *E. coli*
2. to study the potential of CM NSP HP to replace AGP in weaned piglets challenged with *E. coli*
3. to compare the effect of different concentrations of CM NSP HP on piglet performance when challenged with *E. coli.*

2.0 LITERATURE REVIEW

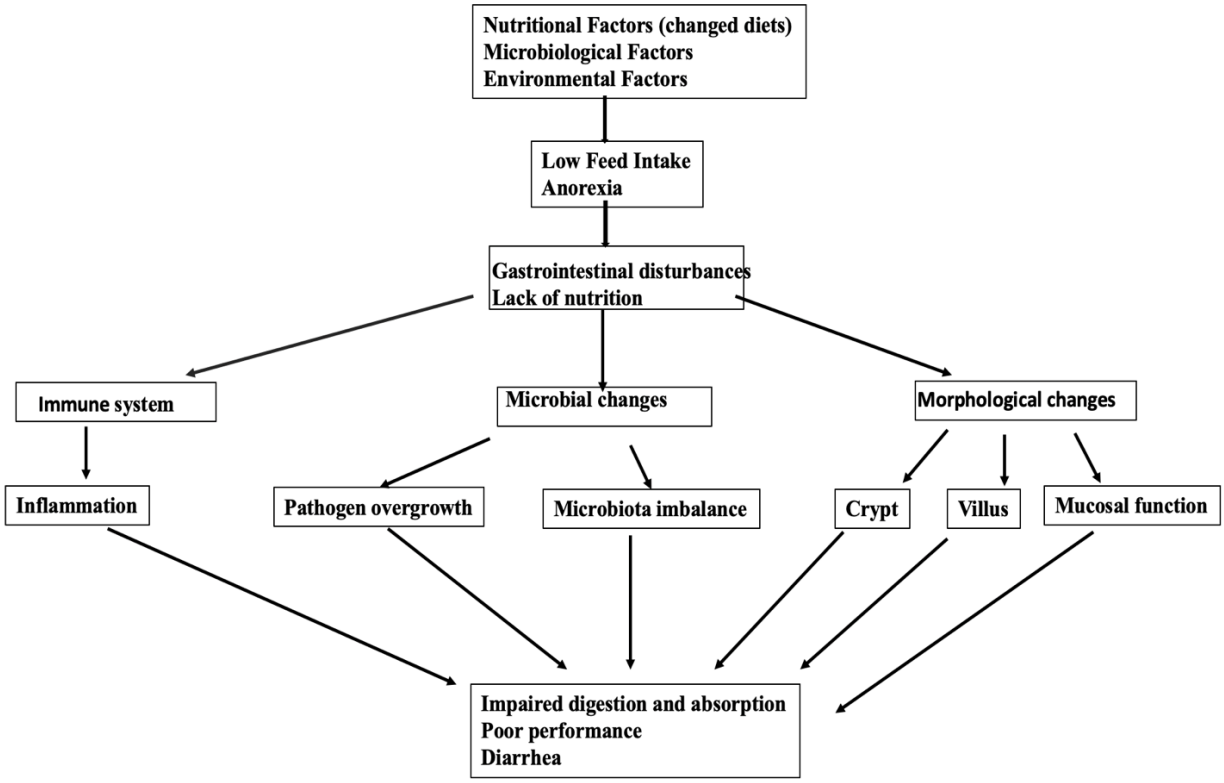
2.1 The Weaning Process and Weaned Piglets

Natural weaning period would happen around 17 weeks old, however, weaning occurs or happens at around 3-4 weeks after birth in the modern swine industry (Gresse et al., 2017). When a pig's diet is suddenly shifted from sow's milk to solids, the pig's intestinal physiology undergoes numerous changes in morphology and microbiota, making them susceptible to post-weaning diarrhea and anorexia, which are associated with disturbances in digestive function, absorption function and immune function (Jayaraman & Nyachoti, 2017). Additionally, piglets must be isolated from the sows and littermates to form new hierarchical subgroups, resulting in social stress. Additionally, the gut microbiota's structure is altered to adapt to the new food and environment (Kluess et al., 2010). As a result, piglets often exhibit decreased feed intake, decreased digestive and absorptive ability, and a high incidence of diarrhea after weaning (Gao et al., 2019). Thus, in order to enhance the growth and gut health of piglets, it is critical to understand the changes in GIT biology of piglets.

2.2 Piglet Gastrointestinal Tract Biology at Weaning

Ggastrointestinal tract (GIT) biology of piglets is also altered throughout the weaning phase as a result of several changes in physiology, microbiology, and immunology (Figure 2.1). It is critical to understand these subtle changes in piglets in order to develop measures to enhance the gut health as well as immune system of weaned piglets.

Figure 2.1 Diagram of piglet post-weaning challenges



2.2.1 Physiological changes at weaning

Ggastrointestinal tract (GIT) which includes all the organs of the digestive system is of relevance to the general metabolism, physiology, disease status and growth performance of pigs during all phases. The GIT is a complex and balanced environment that is impacted by the dietary composition (Everaert et al., 2017). All the organs which are closely related with the system of digestion and absorption of weaned pigs' new weaning diet including the stomach, small intestine as well as large intestine. Piglets adapt to digesting solid food by secreting more acid, altering enzymatic secretion, and adjusting to the rate where the feed passes through the digestive system (Suiryanrayna & Ramana, 2016). However, the most critical and sensitive component of the body during this weaning stage is the small intestine. In the small intestine, many indicators of pig gut

health include villus height, crypt depth, and their ratio. A healthy gut has a high villus to crypt ratio, which is associated with net water and nutrient absorption (X. Wu et al., 2018). Additionally, gut motility and mucous secretions act as a defensive mechanism for the epithelium, moving pathogens and toxins through the gut lumen until they are excreted (Lallès et al., 2004).

Weaning, could also lead to a decrease in nutritional absorption, disease resistance, and performance (Casas et al., 2020). Moreover, deep crypts relate to a rapid turnover of cells while villi shortening may occur as a result of an improved cell loss and a decreased rate of cell regeneration. Villi are constantly regenerated and are susceptible to sloughing, pathogenic attack, and pathogen-induced inflammation. Additionally, inadequate feed intake and nutritional stress may result in impaired gut mucosal integrity, as evidenced by an increase in paracellular transport (Spreeuwenberg et al., 2001). Reduced mucosal integrity is also related with an increased permeability, which may facilitate toxin and pathogen internalization via the epithelium (Brown et al., 2006). Thus, weaning alters the histological and morphological characteristics of the small intestine and has a detrimental influence on its digestive, absorptive, secretory, and barrier functions (Garcia et al., 2016).

2.1.2 Microbiological changes at weaning

Additionally, intestinal microbiota could be changed during weaning period due to the changes of GIT biology of piglets. The gut microbiota performs a lot of functions beneficial to pigs, including carbohydrate digestion and fermentation, vitamin synthesis, intestinal villi maintenance, immune response modulation, and preventing the pathogenic bacteria (Kiarie et al 2010; Kamada et al., 2013). The whole GIT's radial distribution is separated into four microhabitats: the gastrointestinal lumen, the undisturbed mucus layer, the mucous layer in the crypt, as well as the intestinal epithelium's surface (Gaskins., 2000). Due to the stomach's low pH and rapid digestion, there are extremely few bacteria in the stomach (10^3-10^5 CFU/g or mL of contents) and proximal small intestine (Dubeski, 1997). The majority of microbiota present attach to the surface of mucus or epithelial cell, since the pace of digesta flow and bacterial washout over the growth rates of the majority of microbiota in the small intestine (Gaskins., 2001). The distal small intestine, on the other hand, has a greater concentration of bacteria (10^8 CFU/g or ml of contents) (Walter, 2008).

In comparison, there is a greater number of bacteria owing to the digesta's prolonged residence duration in the large intestine (Dubeski, 1997).

During the suckling phase, Lactobacillus and Streptococcus spp. are the dominant microbiota in the stomach as well as the small intestine (Petri et al., 2010). The gut microbiota has been shown to be mainly affected by the composition of sows milk and the genetic makeup of pigs (Bian et al., 2016). One the other hand, the intestinal microbiota is a very complicated ecology with a variable composition and diversity that varies with time and over the whole GIT (Isaacson & Kim, 2012). The gut microbiota is very susceptible to changes in nutrition and social stress, such as those experienced by the weaned piglet.

At weaning, pigs' diets are abruptly modified to include cereals and a high crude protein content. The majority of research has shown an increase in bacteria such as *Clostridium* spp., *Prevotella* spp., or *Escherichia coli*, but a reduction in the *Lactobacillus* group and bacterial diversity (Konstantinov et al., 2006; Mach et al., 2015). Additionally, as *Lactobacillus spp*. are critical participants in preventing diseases, their loss might result in a sudden rise in illness risk during the weaning transition (Konstantinov et al., 2006). During the weaning phase, the reduction in gut bacterial diversity causes the glycans to be more accessible to harmful bacteria. And glycans form the mucus layer covering the gut epithelium (Bäumler & Sperandio, 2016). Additionally, commensal bacteria breakdown of mucus polysaccharides may result in the release of fucose, galactose, and mannose, which may aid pathogenic species in their proliferation. Thus, alteration of the gut microbiota and perturbations of the gut microbial environment are significant risk factors for post-weaning diarrhea.

2.2.3 Immunological changes at weaning

When evaluating the GIT's functioning, the immune system is also a critical factor. Associations between the epithelium, exterior mucous layer, as well as the immune system comprise the gut defence system. In addition, epithelium, which is composed of epithelial cells, tight junctions between cells, and the epithelium's basal membrane, is a border of the lumen and the lamina propria (Gaskins, 2001). In pigs, the external mucous layer generated by goblet cells serves as a protective,

lubricating, and transport layer (Gaskins, 2003). Additionally, the GIT immune system is composed of structured lymphoid cells and mesentery lymph nodes, as well as lymphocytes that are distributed throughout the tissues of the lamina propria and gut epithelium (Stokes et al., 2004). Organized lymphoid cells are consist of multiple follicles (B-cells) and interfollicular zones (T-cells). And plasma and mature cells, are mainly located in the crypts of pigs, whereas T-cells (CD4+ and CD8+) are mostly in jejunum villi (Olivier et al., 1994). In addition, pig intestines also release pro-inflammatory cytokines, which are involved in the cell-mediated immune response and tissue integrity maintenance (Xiao et al., 2014). These immune system components operate in concert to defend the host against harmful pathogens and illness.

Piglets do not have a fully established and mature immune system at weaning, as studies have shown that the immune system of the weaned piglets GIT including immune cells and the whole structure require at least seven to nine weeks to develop in a highly regulated sequence (Bailey et al., 2005). It is revealed that various T-cell populations were enhanced in the lamina propria (King et al., 2003). Additionally, alterations in immunological responses occur shortly after weaning increased the concentrations of pro-inflammatory cytokines in the plasma and glucagon, as well, the increased relative gene expression of pro-inflammatory in the jejunum tissue was showed in the results (Pie et al., 2004).

Two hypotheses exist to account for these alterations in the immune system and inflammation. To begin, anorexia weakens the barrier of epithelial, enabling the antigens to permeate. Second, the immune system is immature during the weaning period, lacking the capacity to discern between hazardous and harmless antigens, and hence exhibits overreaction (Stokes et al., 2004). Anorexia, in particular, increases paracellular rather than transcellular permeability, and a negative link has been shown between villus height which is relevant to the epithelial injury and the numbers of CD4+ and CD8+ cells (Kiarie, 2008; Lallès et al., 2004).

When weaned piglets encounter social stress and are changed to a new diet, gut morphology and function are impaired, and immune responses are significantly amplified (Boyer et al., 2015; Scharek-Tedin et al., 2013). It has been shown that crowding stress during the weaning phase has a direct effect on the immunological response, as evidenced by increased serum cytokines (i.e.,

TNF-a) and plasma cortisol levels (Khafipour et al., 2014; Oh et al., 2010). Additionally, inflammation caused by the stress of weaning has a significant effect on the gut (Pie et al., 2004). And a study has shown that chronic social stress in weaned piglets had a detrimental effect on growth performance, gut barrier integrity, as well as the nutrient transport function (Li et al., 2017). Therefore, the sequence of post-weaning events may create a real vicious circle, resulting in intestinal illnesses.

2.3 Post-weaning Diarrhea

During the weaning period, post-weaning diarrhea (PWD), which relates to GIT disturbances is regarded as a major health issue in the pig business and causes significant morbidity and death (Dadi et al., 2020). The PWD increased the number of mortality and morbidities in pigs, as well as the low pigs productivity and the costs of treatment regimens, resulting in losses for the farmers (Bogere et al., 2019). In the swine business, *Salmonella* enterica *serovar Typhimurium* (*S. Typhimurium*) and Enterotoxigenic *E. coli* are the two most common infections that cause post-weaning diarrhea, resulting in significant economic loss (Heo et al., 2013). In piglets, the ETEC strain is the most frequent and harmful form of bacteria. ETEC is also a bacterial disease that causes PWD in the GIT during the weaning stage and is the primary cause of 50 percent of piglet mortality worldwide each year (Heo et al., 2013).

The genetic background of piglets is a significant factor in the development of intestinal pathogens and is also a significant genetic factor in the development of diseases in pigs. Weaned piglets have recently been shown to exhibit ETEC F4+ and ETEC F18+ pathogenic E. coli-high susceptibility phenotypes, which has prompted an investigation into their origins (Luise et al., 2019). Besides, the gene polymorphism of *Mucin 4* in the pig genome contained within a specific generated ETEC $F4^+$ receptor, thereby protecting pigs against ETEC $F4^+$ infections (Sinha et al., 2019). Based on *Mucin 4* gene polymorphism, those weaned pigs which are susceptible to ETEC have been found to reduce gut bacterial diversity in the gut when compared to non-sensitive pigs (Messori et al., 2013). The lower diversity of intestinal microbiota has been shown related with the increased immune-mediated diseases (Martinez et al., 2015).

In the first week of post-weaning, ETEC F4+ infections in pigs are most common. When the increased prevalence of ETEC-associated diarrhea has been observed in piglets, ETEC F4+ strains have been shown to be the most frequent form of bacterial infection in the piglets (Fairbrother et al., 2005; Zanello et al., 2011). A considerable number of ETEC has been identified in the small intestine, although other bacteria only showed minor modifications in pigs presenting PWD signs (Hopwood & Hampson, 2003). Furthermore, ETEC colonized in the small intestine, and they also create enterotoxins in the GIT, which make epithelial cells to leak fluid in the GIT and change the gut environment of weaned piglets, resulting in the development of diarrhea (Read et al., 2014).

2.4 Strategies to Protect Against PWD

Many suitable strategies have been studied to improve growth performance and GIT ecology, therapy protecting weaned piglets against PWD.

2.4.1 Antimicrobial growth promoters

Antibiotic growth promoters (AGPs) have been chosen commercially as the effective feed additives in the pig industry for many years to decrease the pathogenic bacteria, and stimulate the growth and feed efficiency in weaned piglets (Heo et al., 2013; John, 2013). Although the mechanism of action of AGPs are complicated, it has been suggested a variety of mechanisms for AGPs, all of which are based on direct antibiotic action on the microbial makeup and activity of the intestinal microbiota, which includes both pathogenic and commensal bacteria that compete for nutrition with the host (Brown et al., 2016; Niewold, 2007). As a result, increased pig performance might result in a combination of decreased overall intestinal microbiota and eradication of harmful pathogenic (Heo et al., 2013).

The use of AGPs, on the other hand, is becoming more restricted owing to the probable development of bacterial antibiotic resistance. This is because the extended and illogical usage of AGPs in the food chain has been shown to result in bacterial resistance in both the animals and the people who consume the products from animals. As a result of this resistance, the effectiveness of beneficial medicines has been hampered, resulting in an increase in morbidity and mortality (Aarestrup, 2015; Daudelin et al., 2011). Because the use of antibiotic molecules in animals have

a general similarity to antibiotic molecules used in humans, the potential development of antibiotic resistance in humans is a common concern (Liao & Nyachoti, 2017). Therefore, the use of AGPs has been banned or restricted in various parts of the world (Van Boeckel et al., 2019). European Union banned AGP use in 2006, while in the United States, which has only approved a few numbers of antibiotics after receiving permission from the Food and Drug Administration (FDA) in 2019 (Kátia Maria et al., 2021). In Canada, the use of antibiotics in the animal industry must meet the standards of Health Canada's strict for human and the safety for animal (Landers et al., 2012). Furthermore, the manufacture of new antibiotics for the market has been halted in recent years. In response to this chain of events surrounding the use of antibiotics, researchers set out to develop alternatives that may be used in pig diets to protect weaned piglets from PWD, increase feed efficiency, stimulate growth, decrease odour, and eventually provide health advantages to pigs and their owners (Vondruskova et al., 2010). As a result, it is vital to investigate alternate or replacement techniques for AGPs.

Various alternative elements have been proposed and used in pig diets, with varying degrees of success. Natural replacement methods that have been employed such as organic acids, probiotics and prebiotics, enzymes, and medium chain fatty acids. Other molecules that have been used include essential oils and yeasts, zinc, and plant extracts, as listed in Table 2.1. All of them have been shown to be potential alternatives or replacements to antibiotics in animal trials (Vondruskova et al., 2010). Although there are many other alternatives to antibiotics, probiotics have been commonly utilised as a superior substitute for the subtherapeutic antibiotic dosages that are common chosen in the treatment of PWD (Hou et al., 2016).

Table 2.1 List of proposed AGPs alternatives for weaned pigs

Alternatives	Observations	References
β-glucans	Boosted immune response against *E. coli* infection	Kim et al. (2019)
	Improved the growth performance and alleviate the inflammation response of pigs after LPS administration	C. Wu et al. (2018)
	Increased nutrient digestibility and decreased coliform bacteria in weaned pigs	Park et al. (2018)
Essential oils	Increased the jejunum and ileum villus height	Su et al. (2020)
Manno-oligosaccharides	Decreased apoptosis rate in the jejunal epithelium	Yu et al. (2021)
Chito-oligosaccharides	Greater concentrations of plasma IGF-I in pigs challenged with *E. coli*	Liu et al. (2010)
	Improved intestinal bacteria profile	Wang et al. (2016)
	Tended to increase feed efficiency and improve gut morphology of jejunum in weaned pigs	Lee et al. (2021)
Organic acids	Weaned pigs fed with Microencapsulated organic acids and essential oils supplementation had a higher VH in the mid-jejunum than no-feed additive pigs and	Choi et al. (2020)

	showed anti-diarrhea effects in weaned pigs challenged with ETEC	
Prebiotics	Enhanced serum IgM, IgG, and IgA responses to *S.* Typhimurium infection	Naqid et al. (2015)

2.4.2.1 Probiotics

Probiotics are live microbes that, when consumed in sufficient quantities, may provide a health benefit to the individual ingesting them (Hou et al., 2016). According to Gagga et al. (2010), probiotics have been demonstrated to enhance development in farm animals as well as improve the efficiency of feed consumption, alter the gastrointestinal ecology, boost the immune system, and protect the host against illnesses of the GIT. Thus, probiotics may be used as an alternative therapy to antibiotics (Cho et al., 2011).

A number of probiotics have been studied in pigs and used to prevent PWD and improve the health with positive results (Table 2.2). Whether a microorganism or a mixture of different species of microorganism have been used as probiotic in the pig industry such as lactic acid bacteria (*LAB*), *Bifidobacterium* or *Bacillus* (Bogere et al., 2019).

Table 2.2 A summary of probiotic supplementation in weaned pig diets

Probiotic	Additives	Observations	References
LAB	*Lactococcus lactis* subsp. *lactis* CECT 539, *Enterococcus faecium* CECT 410 and *Carnobacterium piscicola* CECT 4020	Decreased faecal coliform counts of weaned piglets	Guerra et al. (2007)
	Pediococcus. *acidilactici* FT28	Improved crude fibre digestibility	Dowarah et al. (2018)
Bifidobacterium	*Bifidobacterium lactis NCC2818*	Decreased IgA and IgM production by mucosal tissues	Lewis et al. (2013)
Bacillus	2.0 × 10^6 CFU of *Bacillus subtilis* QST713/g	*Lactobacillus* spp. was not observed in the feces of weaned piglets challenged with *E. coli* on days 17 and 19	Tsukahara et al. (2020)
	1.26 × 106 CFU of *Bacillus subtilis*	Increased the fecal score and villus mitotic index in ETEC challenged weaned piglets	Luise et al. (2019)
	1.2 × 10^9 CFU of *Bacillus subtilis* direct-fed microbial	Decreased the fecal diarrhea at 24h post-challenge with ETEC F4	Bhandari et al. (2008)

In a field study, *Enterococcus* faecium were shown to prevent ETEC K88+ adhesion to the mucus membrane in the GIT of weaned piglets. Additionally, probiotics have the ability to modulate the balance of intestinal microbiota by enhancing the activity of digestive enzyme, enhancing the activity of digestion and absorption of diets, feed digestibility, and nutrient efficiency resulted in lower morbidity and mortality, as well as increased performance of pigs (Vondruskova et al., 2010). An additional study found that feeding a probiotic mixture of *Lactobacillus* plantarum and

Lactobacillus reuteri at a concentration of 0.1% (1×10^9 CFU/kg), increased the number of *Lactobacillus* in faces, decreased the fecal diarrhea, or reduced the amount of in digesta of weaned pigs (Zhao & Kim, 2015). From birth through weaning, it has been shown that daily treatment of piglets orally fed with *Enterococcus* faecium DSM 10663 NCIMB 10415 (EcF) at a dose of 1.26 $\times10^9$ CFU twice per day decreased the proportion of piglets with diarrhea and enhanced their average daily gain (Zeyner & Boldt, 2006). In contrast, when diarrhea was present, a solution of glucose-based containing an extra from 2.9 to 5.8 $\times10^8$ CFU of EcF had no therapeutic benefits when diarrhea was present. The reduced diarrhea scores and the percentage of live piglets who had diarrhea after EcF supplementation, on the other hand, were believable enough to reach the conclusion that the addition of probiotic helped balance the general environment in the GIT, which resulted in an increased daily weight gain later in the piglets.

2.4.2.2 Mode of action of probiotics

Many researchers have identified possible methods through which probiotics exert their benefits on the host (Bajaj et al., 2015; Bogere et al., 2019; Boirivant & Strober, 2007; Brown, 2011). Probiotics descend the presence of gastric secretions down the upper intestinal tract, thus, probiotics' capacity to exert their effects on the swine gut is heavily reliant on their ability to withstand these substances and they can withstand the bile secretions as well as the gastrointestinal and they transit down the proximal intestine tract of host animals (Bajaj et al., 2015). The important target locations for probiotic action are the caecum and colon in piglets because they contain varied and dense populations of microorganisms. As a result, once probiotics are taken, they may have an impact on the balance and activity of the intestinal microbiome (Bogere et al., 2019). In theory, probiotics may enhance the health of the host by boosting the number of commensal microbiota in the gut. However, this has not been shown (Bogere et al., 2019). Regardless of the fact that the precise mechanisms of action of probiotics on the GIT of weaned piglets are still needed to be further studied (Wohlgemuth et al., 2009), several plausible mechanisms have been proposed and investigated, including modulating of the gut microbiota, influencing on the ability of nutrients digestion and absorption, modulating of the immune system of pigs, secreting of antimicrobial compounds, as well as the reduction of diarrhea (Bajaj et al., 2015; Brown, 2011; Do et al., 2017;

Sánchez et al., 2017). Also, the processes of some probiotics may be exclusive to a particular strain (Boirivant and Strober, 2007).

2.4.3.1 Prebiotics

Known as prebiotics, nondigestible carbohydrates are known to be specifically fermented dietary nutrients because they are helpful to modulate the composition of gut microbiota and the activity of the gastrointestinal microbiota, which in turn has a favorable physiological impact on the host (Ferreira-Lazarte et al., 2021). As of 2015, prebiotics are becoming more widely described as any sort of dietary item which has a favourable direct or indirect influence on the beneficial microbiota in the GIT, the homoeostasis, as well as inhibiting pathogenic infections (Hutkins et al., 2015).

The majority of prebiotics are classified as non-starch oligosaccharides, including fructooligosaccharides (FOS) and galactooligosaccharides (GOS). These oligosaccharides are created either by the extraction of naturally existing fructans from dietary sources and regulated hydrolysis of the fructans, or through the transglycosylation of sucrose (Kiarie, 2008). Short chain fatty acid (SCFA) which can produce beneficial bacteria in the GIT including the butyrate-producing bacteria, may be stimulated, allowing them to deliver substrates to intestinal cells while also promoting proper proliferation and differentiation in the cells (Fouhse et al., 2016). It has been studied the potential effect of prebiotic on weaned pigs as shown in Table 2.3. Studies on weaning piglets have revealed the effects of prebiotics on the gut microbiota, including an increase in the amount of *Lactobacillus* and a decreased proportions of harmful bcateria including *Clostridium* and *Enterobacteriaceae* (potentially O'Doherry et al., 2010; Jiao et al., 2014). However, no research has been shown the statistically impact of prebiotics on the amout of ETEC K88 or *S. Typhimurium* in the digesta of weaned pigs which have been orally challenged (Pieper et al., 2012; Thomson et al., 2012; Guerra-Ordaz et al., 2014). Research in the future should validate the possible importance of prebiotics in the treatment of the weaning period and explain the particular effect of the numerous fibres that are presently accessible.

Table 2.3 A summary of prebiotic supplementation in weaned pig diets

Prebiotic	Additives	Observations	References
β-glucan/mannan-oligosaccharides	0.2% of β-glucan/mannan-oligosaccharides addition in diets	Decreased the diarrhea incidence and intensity of weaned piglets	Silva et al. (2020)
	Pediococcus. *acidilactici* FT 28	Improved crude fibre digestibility	Dowarah et al. (2018)
Feed ingredients	Wheat bran, Casein-glycomacropeptide and exopolysaccharides extration	Decreased the number of ETEC on intestinal mucus *in vitro*	González-Ortiz et al. (2014)
Yeasts products	Yeast products (hydrolyzed or non-hydrolyzed) derived from *Kluyveromyces fragilis*	Phagocytic activity of monocytes was higher in both yeasts supplemented groups	Keimer et al. (2018)
	Yeast-derived mannan-rich fraction	Reduced relative abundance of *Sutterella* and *Prevotella*	Fouhse et al. (2019)

2.4.3.2 Mode of action of prebiotics

A study found that prebiotics may modify the gut microbiota by regulating helpful bacteria groups such as *Lactobacillus* (LAB) and *Bifidobacteria* by supplying food for bacteria and limiting undesirable intestinal colonization of pathogenic bacteria (Hajati & Rezaei, 2010). Instead of being digested and absorbed in the upper GIT, prebiotics function as a food supply for beneficial bacteria in the digestive tract. This ultimately prevents infections such as Salmonella from attaching to the intestinal mucus and stimulates the growth of beneficial bacteria in the GIT. Some sugars have the ability to prevent pathogens from adhering to the mucosa, hence increasing the integrity of the gut mucosa (Adhikari & Kim, 2017). Examples include mannooligosaccharides (MOS), which bind to the mannose-specific lectin found in gram-negative bacteria that express Type-1 fimbriae, such as E. coli, and cause their expulsion from the colon (Thomas et al., 2004). Previous study showed that MOS may affect the immune system and help to eradicate germs from the tract (Tiwari et al.,

2020). GOS have been proven to enhancing the profile of some beneficial bacteria such as *LAB*, *Bifidobacteria*, as well as the fermentation products produced by these bacteria (Macfarlane et al., 2008). As a byproduct of the fermentation process, SCFAs, namely butyrate, propionate, and acetate, are produced. These SCFAs, which decrease the pH of the gut lumen while also providing energy to epithelial cells, are one of the primary mechanisms of prebiotics (Pourabedin & Zhao, 2015).

2.5 Canola Meal

Canola meal (CM) is a by-product of the canola seed industry, and it is commonly utilized for the extraction of canola oil for human use. Because of its low cost and high nutritional content compared to other protein sources, CM has been commonly utilized in swine diets for many years. It is *Brassica napus* that is the most widely planted canola species in the world, whereas *Brassica juncea* is a recently produced variety that is grown in warmer and dryer portions of the Canadian prairies. Canola meal also has a significant amount of dietary fibre and glucosinolates (Khajali & Slominski, 2012). The fiber in CM is divided into soluble and insoluble fractions based on the solubility of NSP. As shown in Table 2.3, canola meal contains more fiber than soybean meal (SBM), however, the fiber in CM is highly lignified and poorly fermented compared with the fiber in SBM or resistant starch. It has been shown that CM includes a high concentration of insoluble fibres and it helped to accelerate the digesta retention time in the GIT, reducing the proliferation of pathogens or bacteria (Hong et al., 2020; Molist et al., 2014). This finding means CM fiber might be closely relevant to protect piglets against PWD (Molist et al., 2014). Researchers discovered that the glucosinolates from CM exhibited antibacterial as well as the antioxidant activity *in vitro* studies (Hong et al., 2020; Yadav et al., 2021). As a result, numerous researchers are investigating the effects of canola meal on pigs' performance, immunological response, the composition of gut microbiota, and gut integrity barrier.

Table 2.4 Carbohydrate (g/kg DM) and NSP contents (% DM) in common feedstuffs[1]

Ingredient	Carbohydrate	Total NSP	Major NSP
Corn	830	11.9	Cellulose
Wheat	814	7.8-12.9	Arabinoxylans, xyloglucan
Canola meal	454	21.9	β-1, 4 galactan
Soybean meal	400	14.8	Galacturonans, arabinans, galactomannan
Barley	823	16.7	Mixed-linked β-glucan

[1]Partially adapted from Cheng et al. (2014) and Adewole et al. (2016).

2.6 Anti-Nutritive Effects of Non-Starch Polysaccharides

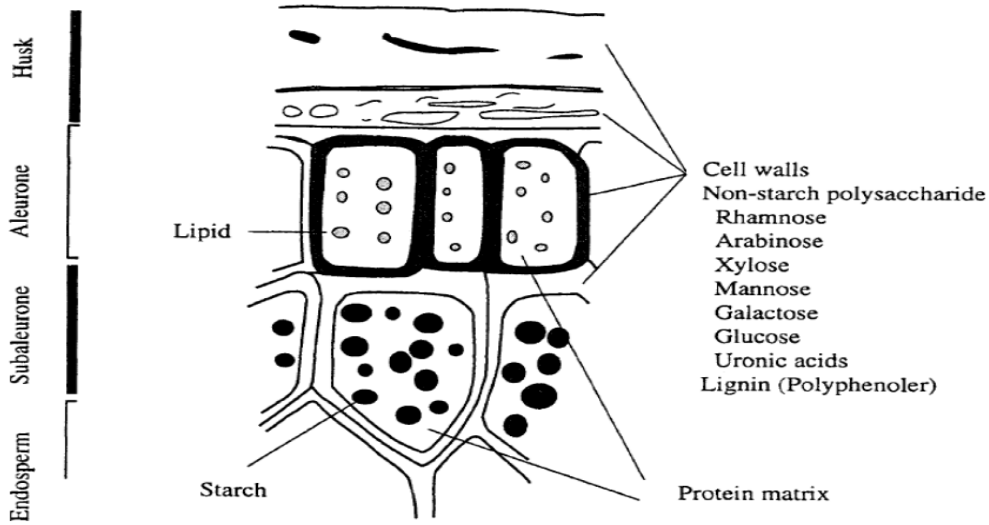
Among the carbohydrates in canola meal, simple sugars, starch, and dietary fibre (DF) are among the most prevalent types. Only simple sugars are capable of being absorbed directly in the small intestine. Endogenous enzymes are susceptible of hydrolyzing starch, leading to the release of their monosaccharides due to the process in the small intestine (Bach Knudsen, 2001; Slavin, 2013). Dietary fibre is made up of NSP and lignin, however, both of which are refractory to endogenous enzymes in the host. Non-starch polysaccharides (NSP) are the most abundant kind of fibre and comprise various polysaccharide molecules, which are typically in conjunction with various lignified polymers, protein, and starch, and which contain glycosidic linkages different than the α - (1-4) and (1-6) bonds found in starch (Bach Knudsen, 2001; Slavin, 2013).

While CM contains significant quantities of NSP (17-22%), these polysaccharides were labelled as "anti-nutritive factors" because of the unfavorable impact on energy and nutrient usage (Kiarie, 2008). This is because CM NSP are poorly soluble in water and hence cannot be digested by pigs, due to lack of endogenous enzymes suitable for NSP digestion. Furthermore, because of the intricate interactions between NSP and the gut epithelium, mucus, and bacteria, NSP have been shown to have detrimental impacts on pig nutrient digestibility, endogenous loss, according to a large number of research studies (Paternostre et al., 2021; Pluske et al., 2002; Yang & Zhao, 2021).

Generally, NSP are known to enhance the intestine's transit time, delay stomach emptying and glucose absorption, boost pancreatic output, and reduce absorption (Pluske et al., 2002).

Cellulose (linear -glucan chains), non-cellulosic (arabinoxylans, mixed-linked -glucans, mannans, galactans, or xyloglucan), pectic polysaccharides (polygalacturonic acids), as well as fragrant polymer lignin are the principal NSP of canola cell walls. (Mejicanos, 2019; Pluske et al., 2002). The hard physical structure of NSP (Figure 2.2) is a key aspect in the plant cell wall's ability to resist breakage (Liu et al., 2020). As a consequence, CM cell walls can protect against biochemical degradation and physical damage, which may have an impact on the digestion and absorption of NSP in the body.

Figure 2.2 The cell wall structure of canola meal (Bach Knudsen, 2001)



2.7 Carbohydrase Digesting Non-Starch Polysaccharides

Utilizing carbohydrase enzymes to target NSP in the cell walls is a typical method of increasing CM usage (Khajali & Slominski, 2012). Cell wall polysaccharides would be hydrolyzed more efficiently if NSP-degrading enzymes were present, decreasing the anti-nutritive effect of cell walls and enhancing the utilization of protein and other nutrients (Radfar et al., 2017). Non-starch polysaccharides degrading enzymes (carbohydrase or glycosidase) can digest the NSP in feed components. They have been used in diets for monogastric animals for over 30 years, and they have been shown to be effective. Carbohydrase, proteases, and phytase are enzymes originating

from fungi and bacteria that are extensively used in animal feed (Castillo & Gatlin, 2015). Multiple studies showed that the inclusion of xylanase or xylanse-glucanase may mitigate the negative effects of arabinoxylans in wheat middling, and xylanse-glucans in barley, thus boosting the nutritional effects of these grains as well as the performance of monogastrics. The effectiveness of enzyme supplementation to different grain diets inspired research in generating enzyme treatments for other feed components.

The NSP in canola can be broken down by CE, which has been created and employed as a way to extract energy and nutrients from the cell wall NSP in a research by Kiarie (2008). It has been shown that this newly designed CE may depolymerize up to 35% of CM NSP (Radfar et al., 2017). Also, CE has been shown to result in NSP depolymerization of CM both *in vitro* and *in vivo,* and an improvement in nutrients digestibility (Meng et al., 2005). Aside from this, it has been shown that the CE supplementation in CM improved the gain to feed ratio of young birds (Radfar et al., 2017). Thus, it is possible that CE in conjunction with CM in diets improve the energy and nutrients utilization in animals.

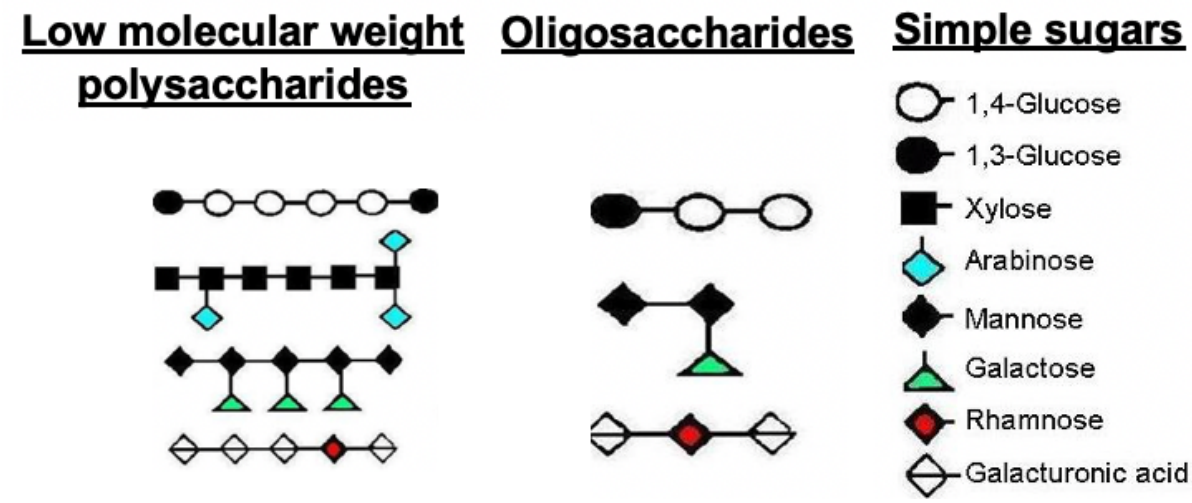
2.8 Mode of Action of NSP-Degrading Enzymes

Several mechanisms might be at play in the favourable response to NSP-degrading enzymes supplementation, according to certain theories. It has been suggested by Simon (1998) that CE directed towards NSP may have several different mechanisms of action. These include partial hydrolysis of NSP, reduction in the viscosity of digesta, breakage of cell walls in NSP-containing cells, and thereby releasing of nutrients from the encapsulated nutrients. The large molecules of plant NSP could be broken down into smaller polymers and oligomers, and a few connections between the backbone of the polymer could be also broken down by some NSP-degrading enzymes (Mathlouthi et al., 2003).Thus, the digesta viscosity which is relevant to the chain length of water-soluble polysaccharides would be reduced (Mathlouthi et al., 2003). Plant cell walls serve a structural purpose and are fundamentally more resistant to destruction than animal cell walls. To disrupt cell walls to a substantial level within a very short period, unlike viscosity reduction, many various types of enzymes must be present (Bach Knudsen, 2001; Navarro et al., 2019).

2.9 Effect of Non-Starch Polysaccharides on Intestinal Microbiota

In the process of digesting the NSP in the diets, enzymes might create a diverse range of NSP HP, each with a distinct sugar component and a different molecular weight. The NSP HP may contain simple sugars, low molecular weight polysaccharides, as well as oligosaccharides (Figure 2.3). Although monosaccharides may be generated because of the enzyme addition, the higher growth performance cannot be considered as the increased simple sugar usage or, to increased energy production (Mikkelsen et al., 2003). Considering the poor absorption and utilisation of certain sugars (such as xylose, arabinose, and uronic acids), it seems that allowing NSP HP to transit into the hind gut of weaned piglets as oligosaccharides and then are fermented or digested by the gut microbiota may be reasonable (Mikkelsen et al., 2003; Tiwari et al., 2020).

***Figure* 2.3** NSP hydrolysis products (Hong et al., 2020; Radfar et al., 2017)



As most microbiota favour carbohydrates as the energy source over protein, saccharolytic fermentation occurs likely in the proximal colon (Qaisrani et al., 2014). However, proteolytic fermentation mostly occurs in the hind gut and could cause the production of potentially dangerous metabolites such as branched-chain fatty acids (BCFA) and nitrogenous metabolites such as amines, ammonia N, as well as indoles (Hamer et al., 2008). Many colonic diseases appear in the distal colon due to the less fermentable carbohydrates and harmful nitrogenous metabolites (Nielsen et al., 2014). Thus, it is desirable to have non-digestible carbohydrates transited into the

hind gut and have the carbohydrates fermentation by microbiota (Kolida et al., 2002). This coincides with the higher growth performance and improved gut health of pigs when NSP are degraded by CE because NSP HP may be delivered to the hind gut and fermented by microbiota (Mikkelsen et al., 2003).

It has been studied that NSP hydrolysis products, such as oligosaccharides, can be partially or completely fermented in the large intestine of weaned piglets by the microbiota, which results in the release of SCFA, such as acetate, propionate, and butyrate (Mengíbar et al., 2013; Slavin, 2013). Furthermore, some hydrolysis products, such as mannan-oligomers, have the potential to draw microorganisms away from intestinal binding sites via the process of competitive exclusion, therefore limiting colonisation and disease development while allowing the mucosa to fulfil its functions of secretion, digestion, and nutrient absorption (Mathlouthi et al., 2003). Thus, NSP HP which are produced during the NSP degrading by CE are closely connected and co-influenced with intestinal microbiota.

2.10 The Prebiotic Effect of Non-Starch Polysaccharides Hydrolysis Products

The hydrolysis products of NSP from cell walls that result from the addition of CE may function as prebiotics and promote the development of lactic acid bacteria, so lowering the proliferation of *Clostridium perfringens* (Kiarie, 2008). These finding are closely consistent with our previous studies both *in vitro* and *in vivo*. Kiarie (2008) demonstrated that enzyme hydrolysis products derived from the NSP of SBM CM improved net absorption of fluid in ETEC-infected porcine segments *in vitro* and helped maintain fluid balance of segments during ETEC injection, suggesting that NSP hydrolysis products may be useful in managing enteric illnesses such as ETEC-secretory diarrhoea. Besides, our previous studied also showed weaned piglets fed with the addition NSP hydrolysis products derived from ethanol-extracted wheat middling, SBM, CM and flaxseed had lower amount of ileal ETEC and digesta ammonia compared with those pigs without feed additives when challenged with ETEC (Kiarie et al., 2010).

Transgalacto-oligosaccharides as a common form of galacto-oligosaccharide, that may have potential prebiotic benefits in weaned piglets. They are produced commercially by the

transglycosylation actions of -glycosidases on lactose, leading to -(1,6) polymers of galactose attached to a terminal glucose unit via a -(1,4) glycosidic bond (Marín-Manzano et al., 2013; Navarro et al., 2019; NRC, 2012). Transgalacto-oligosaccharides, on the other hand, are not produced in nature (Marín-Manzano et al., 2013; Navarro et al., 2019; NRC, 2012). Mannan-oligosaccharides are formed of polymers of mannose that are created from yeast cell walls and are located on the outer surface of yeast cell walls, where they are linked to -glucans of the inner matrix via -(1,6) and -(1,3) glycosidic connections. Because of their good health effects on the host, mannan-oligosaccharides and fructo-oligosaccharides could operate the similar effects with prebiotics by encouraging the development of beneficial bacteria and modulating the microorganisms in the large intestine (Biswas et al., 2021). It haves been suggested that mannan-oligosaccharides as a method of modulating pigs' immune responses to the changed of immunologic and preventing overstimulation of the host animal's immune system following infection (Adewole et al., 2016).

2.11 Summary of Literature Review

Weaned piglets commonly suffer from PWD due to the weak and undeveloped GIT physiology. The GIT is essential for growth and health for weaned piglets because it is closely related to nutrient digestion, absorption, and gut bacteria as well as mucosal immune system. However, PWD could be harmful to GIT physiology, gut microbiota and immune system of weaned piglets. Researchers have studied many methods or strategies to protect against PWD and improve gut health of weaned piglets. Antimicrobial growth promoters (AGPs), as important feed additives are increasingly being eliminated or banned in pig industry. Some alternatives to AGPs that have been studied include organic acids, minerals, prebiotics, and probiotics, and many of these have been shown to be beneficial for controlling enteric diseases and balancing the gut microbiota.

Among these alternatives, one potential non-antibiotic method is to use the dietary components. There is some evidence that dietary NSP could be depolymerized by CE to release HP. Since these NSP HP are not absorbed by the host, however, they have been shown to modulate the GIT microbiota including pathogens in similar functions as prebiotics. Compared with corn, wheat and soybean meal, CM contains higher level of NSP that could be breakdown by CE. Therefore, whether CM NSP HP

could be used as feed additives to improve gut health and immune system of weaned pigs and serve as alternative to AGP needs to be studied.

3.0 MANUSCRIPT

POTENTIAL PREBIOTIC EFFECT OF CANOLA MEAL NON_STARCH POLYSACCHARIDE HYDROLYSIS PRODUCTS IN WEANED PIGLETS CHALLENGED WITH ENTEROTOXIGENIC *ESCHERICHIA COLI* F4

3.1 ABSTRACT

Enzymatically derived non-starch polysaccharide (NSP) hydrolysis products (HP) may modulate enteric health outcomes in piglets. Thus, responses of weaned piglets to an oral challenge with enterotoxigenic *Escherichia coli* F4 (ETEC) when fed diets containing canola meal (CM) HP were investigated. The HP were obtained by incubating CM with a multi-carbohydrase (MC) blend. Thirty-five weaned piglets (8.08 ± 0.34 Kg BW) were individually assigned in a completely randomized design to 1 of 5 treatments: NC (no challenge), CC (challenged control with ETEC), AGP (CC + 0.1% AGP), HP1 (CC + 0.25% HP), HP2 (CC + 0.5% HP). After a seven-day adaptation period, piglets in the NC group received 5 ml of PBS, whereas those in all other groups were orally challenged with 5 ml (5×10^6 CFU/mL) of ETEC F4. On d 14 all piglets were euthanized for tissue sampling. Data were analyzed using the PROC MIXED procedure of SAS 9.4. During the post-challenge period, piglets fed NC diet had lower ($P < 0.05$) G: F than those pigs fed the AGP, whereas the G: F was higher ($P < 0.05$) for piglets fed the HP-containing diets than that of piglets fed the CC diet. Piglets fed diets containing HP showed lower ($P < 0.05$) jejunum pH and fecal scores than piglets fed the CC diet. For gene expressions, the HP2 group showed a lower level of *IL-6*, *TNF-a*, toll like receptor 2 (*TLR2*) and toll like receptor 4 (*TLR4*) compared with the CC group ($P < 0.05$). Results in the HP groups had higher *BCVFA* and *lactobacillus* in the colon digesta compared with those in the CC treatment ($P < 0.05$). In conclusion, piglets fed diets with CM HP exhibited less severe ETEC-enteritis and had similar effects with AGP.

Key words: Non-starch polysaccharides hydrolysis product; Piglets, Post-weaning colibacillosis

3.2 Introduction

Weaning time is a challenging period for piglets as a result of the changed diet from milk to solid diet and changes in intestinal physiology as well as the environmental changes (Heo et al., 2013). Weaned piglets have poor gut function and weak digestive and absorptive capacity of nutrients, therefore, piglets are vulnerable to post-weaning diarrhea (PWD) and weaning anorexia and enteric diseases are common during the weaning period (Jayaraman & Nyachoti, 2017). This is an important factor for piglets growth and health and has been shown to lead an economic loos in the pig industry. Thus, suitable strategies are essential to be studied to improve growth performance and gastrointestinal tract (GIT) system of weaned piglets.

Antibiotic growth promoters (AGPs) have been used to effectively decrease the incidence of PWD and boost the gut health and enhance the growth performance of piglets at weaning. However, long-term use of AGPs in the pig industry has been related with the increased bacterial resistance to antibiotics (; Holmes, 2016), thus, AGPs have been limited or banned to use in many countries (Woolhouse et al., 2015). Therefore, more studies about seeking alternative or replacement strategies for improving growth performance and maintaining gut health for weaned piglets are needed.

Recently, researchers found dietary components could be considered as a non-antibiotic method to replace the use of AGPs (Huyghebaert et al., 2011). Canola meal (CM) has been widely used in swine diets because of relatively lower price and good nutritive values (Mejicanos, 2019). However, CM contains high levels of non-starch polysaccharides (NSP). Furthermore, CM NSP are highly insoluble in water and cannot be digested by pigs because they do not have the endogenous enzymes to digest NSP (Kiarie, 2008; Kiarie et al., 2010). Canola meal NSP can be broken down by carbohydrase enzymes (CE) which have been developed and used as a means to extract energy and nutrients from the cell wall NSP (Kiarie et al., 2010). Carbohydrase enzymes supplementation tended to improve the dry matter (DM) digestibility of CM and total gas and volatile fatty acids (VFA) production on pig in vitro digestion and fermentation characteristics, which substantiated that the CE improved utilization of nutrients via shifting microbial fermentation (Lee et al., 2018). Meanwhile, in the process of digesting CM NSP, hydrolysis products will be released, including simple sugars, oligosaccharides as well as low molecular

weight polysaccharides. Some xylo-oligomers, mano-oligomers, gluco-oligomers may behave as prebiotics (Kiarie et al.,2010). For example, xyo-oligomers, the hydrolysis product of xylanase, could stimulate the growth and activity of beneficial colon, and prevent the growth of pathogenic species (He et al., 2020; Pollet et al., 2012). Our previous study also confirmed that the addition NSP HP of wheat middling, SBM, CM and flaxseed had lower amount of ileal ETEC and digesta ammonia compared with those pigs without feed additive when challenged with ETEC (Kiarie, 2008). According to Long's study (2021), dietary CE supplementation not only improved the digestibility of crude protein and gross energy, but also increased the immunoglobulin and *Lactobacillus* community, which could be explained that the oligosaccharides produced by degraded NSP with enzyme could help improve the immune function in pigs. Therefore, NSP HP can help improve the growth performance and enhance the gut health of weaned pigs and is worthy to investigate the potential prebiotic effect.

Our previous study showed the perfusion of the water-soluble (supernatant) part of NSP HP from wheat middling and flaxseed had higher total solute absorption than water-insoluble part of NSP HP in our previous study. There is no study about the effect of CM NSP HP on weaned piglets, moreover, previous studies used a challenging mode of *E. coli*, the most common pathogens for piglets (Kiarie, 2008; Pluske et al., 2018; Sun & Kim, 2017). Thus, this study used ETEC challenging model to study the effect of CM NSP HP. Therefore, this study was performed 1) to investigate the effect of CM NSP HP on growth performance, gut health and immune system of weaned piglets challenged with ETEC; 2) to study the potential of CM NSP HP to replace AGP in weaned piglets challenged with ETEC; 3) to investigate the effect of different concentrations of CM NSP HP on piglets performance when challenged with ETEC.

3.3 MATERIALS AND METHODS

The protocol of this experiment was reviewed and approved by the Animal Care Committee of University of Manitoba. Piglets were housed and managed by following the introductions and guidelines of the Canadian Council on Animal Care (CCAC, 2009).

3.3.1 Non-starch polysaccharide hydrolysis products

Before incubation with CE, CM samples were incubation with ethanol in order to remove free sugars as well as components of low molecular weight carbohydrate such as sucrose and oligosaccharides as shown in previous study (Slominski et al., 1993). Then, each 150 g of CM was mixed with 600 ml 80% ethanol into the 1L flask and incubated in an incubator shaker set at 200 rpm and 40 ºC for 16 h (New Brunswick Scientific, Inc., Edison, NJ). After incubation, the mix was transferred into plastic bottle (centrifuge insert) and then centrifuged for 15 min set at 2,000 rpm. The supernatant part was decanted and the precipitate was dissolved in 600 ml of 80% ethanol and transferred back into volumetric flasks. Precipitate was mixed in the incubator shaker for 3h at 200 rpm and 40 ºC and then was centrifuged for 15 min at 2,000 rpm. After the centrifugation, the precipitate was dried to remove the ethanol at room temperature within a fume hood overnight.

The second step was to incubate the ethanol-extracted CM with enzyme. The enzyme blend contained Pectinase A (0.4%), Pectinase B (0.4%), Xylanase B (0.4%). The provided enzyme preparations in this study, following with the procedures of enzyme assay by Canadian Bio-System Inc., (Calgary, Alberta, Canada). Canola meal NSP HP were produced by mixing 2 kg of ethanol-extracted CM with 1.22% of CE blend in distilled water and incubated for 16h in an incubator shaker at 200 rpm and 40ºC. After the enzyme incubation, the mixture was centrifuged at 2,000 rpm for 20 min and get the supernatant part. The supernatant and water-soluble was frozen at -80°C, then freeze dried, finely ground, and stored at 4°C for future study.

3.3.2 NSP hydrolysis product analysis

The carbohydrate contents of CM NSP HP were measured by GLC to analyze the component of neutral sugars and uronic acids was measured by using colorimetry (Slominski et al., 2006). Initially, 30 mg of CM NSP HP was combined with 6 mL of distilled water and was combined with 5 mL of mononitride solution and 1 mL of 12 $mol \cdot L^{-1}$ sulfuric acid before being subjected to two hours of boiling. Analysis of the hydrolysate combination reported the existence of uronic acids and neutral sugars as neutral sugar components. Then, 1 mL of hydrolysate was neutralised with 12 mol/L ammonium hydroxide, decreased with sodium borohydride, as well as acetylated with acetate anhydride in the presence of 1-methylimidazole to create the component neutral

sugars. The sugar components were measured using an SP-2340 column and a Varian CP 3380 gas chromatograph, respectively (Varian Canada) (Varian Canada). Besides, uronic acids were measured by the procedure described by Scott (1979).

3.3.3 Gene screening and piglet selection

The ETEC F4 sensitive pigs were chosen in accordance with the procedures (Jensen et al., 2006). Tails of piglets were collected on the age of day3 and the DNA of piglets was extracted following a technique reported by Truett et al., (2000). Firstly, the amplification of MUC4 gene was carried out in a 25 μL mixture with the method of DreamTaq DNA polymerase (Thermo Fisher Scientific) including 2 $mmol \cdot L^{-1}$ $MgCl_2$, 200 $\mu mol \cdot L^{-1}$ of each dNTP, and 400 $mol \cdot L^{-1}$ of each primer utilizing a total of 2 $mmol \cdot L^{-1}$ $MgCl_2$. Thermocycling was carried out utilizing a 5 minutes initial denaturation at 95°C, followed by 30 s at 95°C, 30 seconds at 65°C and 1 minute at 72°C for 35 cycles. 367 base pairs (bps) were got from pig genomic DNA, and 5 μL of the PCR products were digested by FastDigest XbaI (Thermo Fisher Scientific) at 37°C for 5 minutes, as directed by the supplier. After being digested, the products of PCR procedure were electrophoresed on a 2% agarose gel in a Tris-borate-EDTA solution and stained with SYBR Green to determine their identity (Invitrogen). Piglets with vulnerable gene and comparable body weight (BW) were chosen in this study.

3.3.4 Preparation of ETEC F4

The ETEC F4 strain was cultivated anaerobically overnight at 37°C on tryptic soy agar from frozen stock. The single ETEC F4 colonies from the streak plate then injected into 10 mL of tryptic soy broth (sterile) and allowed to develop aerobically overnight at 37°C in a shaker incubator (MaxQ SHKE4000; Thermo Fisher Scientific) set at 150 rpm till the next day. The culture was inverted at 45°C to allow sufficient aeration. Then after, 300 mL of the overnight culture was used as a bacterial suspension for a new 300 mL of tryptic soy broth (sterile), which was maintained at 37°C and stirred at 150 rpm with the use of an orbital shaker again. The culture was allowed to develop for 2.5 hours. Once constructing a growth curve and a standard curve, the final ETEC F4 inoculum was prepared after the necessary preparation experiments were performed. After incubation with a Pharmacia Ultrospec 2,000 spectrophotometer (Pharmacia Biotech, Cambridge, UK), the

bacterial density was estimated using an OD measurement at 600 nm (tryptic soy broth was used as a blank) in accordance with the previously created standard curve. To achieve the appropriate ETEC F4 concentration (5 X10^6 CFU·mL^{-1}) diluents such as phosphate buffered saline (PBS; pH = 7.4) were used. When the culture was brought to the location for inoculation, it was done so using ice packs.

3.3.5 Animals, housing, and experimental design

Thirty-eight female piglets weaned at 28 d (3 spared pigs; TN Tempo × TN70; 8.08 ± 0.34 kg of average BW) which were sensitive to ETEC F4 were chosen from the Glenlea Swine Research Unit. Piglets were separately housed in a temperature-controlled room in T.K. Cheung Centre at the University of Manitoba. Pigs were randomly assigned to individual pens (n =1 piglet per pen). Piglets were divided into 5 treatments to give 7 replicates per treatment. Following a seven-day adaption period, piglets in the NC group were given 5 mL of PBS, while those in the other groups were given 5 mL (5 106 CFU·mL^{-1}) of ETEC F4. Piglets were allowed free access to water and feed from 1 to 14 d. Room temperature was maintained at 29 ± 1°C from 1 to 7 d and reduced by 1.5°C during the second week.

The 5 experimental diets were based on corn because there are low concentrations of NSP or oligosaccharides in corn. The treatments included: NC (no challenge), CC (challenged control with ETEC), AGP (CC + 0.1% AGP), HP1 (CC + 0.25% HP), HP2 (CC + 0.5% HP). All diets were formulated to meet the NRC (2002) nutrient specifications for pigs weighing 5 to 10 kg. The AGP was Aureomycin (Zoetis Canada Inc., Kirkland, QC, Canada).

Following a 7-day adaptation period, piglets in the NC group were given 5 ml of PBS, whereas those weaned piglets in other 4 challenged groups were orally challenged with 5 ml (5 × 10^6 CFU·mL^{-1}) of ETEC F4 by a syringe attached to polyethylene tube held into the upper esophagus (B. Koo et al., 2020; Koo et al., 2017). On day 7 and day 14, the BW of each piglet and feed loss were recorded. During the challenged period, fecal score (Marquardt et al., 1999) of each piglet was recorded every day by 0-3 (0 = well-formed feces; 1 = soft feces; 2 = fluid feces; 3 = liquid feces). On d 14 all piglets were euthanized for tissue sampling.

Table 3.1 Ingredient and calculated nutrient composition of basal diet (%, as-fed basis)[1]

Item	(%)
Ingredient	
Corn	37.80
Wheat (CP 13%)	20.00
Soybean meal (CP 46%)	22.50
Fish meal	5.00
Dried whey	10.00
Vegetable oils	0.50
Limestone	1.00
Monocalcium phosphate	0.40
Salt	0.40
Vit-Min premix	1.00
Lys-HCl	0.48
DL-methionine	0.19
L-threonine	0.16
L-tryptophan	0.02
L-valine	0.05
Total	99.5
Calculated Nutrient, %	
NE, kcal/kg	2,456
CP, %	21.20
Ca, %	0.80
SID[2] Lysine, %	1.35
SID Methionine, %	0.48
SID Threonine, %	0.79
SID Tryptophan, %	0.23

[1]The diet for control was prepared by adding 5 g·kg^{-1} of corn-starch in the basal diet. The diet for AGP was prepared by adding 1 g·kg^{-1} of Aureomycin 220G (Zoetis Canada Inc., Kirkland, QC, Canada) and 4 g·kg^{-1} of corn-starch into the basal diet. The diet for HP1 was prepared by adding

2.5 g·kg^{-1} of CM NSP HP and 2.5 g·kg^{-1} of corn-starch; HP2 was by adding 5 g·kg^{-1} of CM NSP HP in the basal diet.
[2]Standardized ileal digestible amino acids.

3.3.6 Tissue and digesta sample collection

On day 14, all piglets were sedated with ketamine: xylazine (20:2 mg·kg^{-1}BW) and then all piglets were euthanized using a captive bolt gun. Immediately after, the abdomen was cut and opened in order to expose the whole GIT system and get the samples. An 8-cm section of the mid-jejunum was removed and immediately placed in cold Krebs ringer buffer (KRB; in mmol· L^{-1}: 154 Na^+, 6.3 K^+, 137 Cl^+, 0.3 H_2PO_4, 1.2 Ca^{2+}, 0.7 Mg^{2+}, 24 HCO_3^- – pH 7.4 with 1 μmol· L^{-1} of indomethacin), After removing another 10 cm piece of the mid-jejunum, it was promptly snap-frozen in liquid nitrogen and preserved at –80°C awaiting further analysis. An 8-cm slice of the mid jejunum was taken and preserved in a 10% formaldehyde solution for the purpose of determining gut morphology measurement. Jejunum and colon digesta were taken and promptly frozen in liquid nitrogen before being stored at –80°C for further examination.

3.3.7 Digesta pH measurements

The pH of the jejunum and colon digesta were measured on digesta samples. All pH measurements were made by using an electronic (Accumet Basic, Fisher Scientific, Fairlawn, NJ). And pH meter was standardized with certified pH 4 and 7 buffer solutions to keep the accuracy.

3.3.7 Ammonia N and organic acids

The ammonia N concentration in the jejunum digesta and faeces was determined using the method published by Novozamsky et al (1974). In brief, 5 g of jejunum digesta or feces were diluted with 0.1 N HCl (1:5, wt/vol), vortexed, and centrifuged at 2,000 x g for 10 minutes. After that, the supernatant (50 μL) was transferred to a 10-mL test tube and vortexed with 1.5 mL of a solution containing 200 mL of 0.05% sodium nitroprusside and 10 mL of 4% EDTA. And then, a solution containing 10 % NaOCl (2.5 mL) was added to the mixture and vortexed. Test tubes containing the final mixture were placed on a test tube rack wrapped in black plastic sheets and placed in full

darkness for 30 minutes before being tested. Test tubes containing the final combination were placed on a test tube rack wrapped in black plastic sheets and kept in full darkness for 30 minutes before being measured for absorbance at 630 nm. The concentrations of ammonia-N were calculated using a regression equation of the standard curve (25 to 200 $mg \cdot L^{-1}$). To obtain the final ammonia concentrations in the sample, the standard curve values were corrected for dilution.

The concentrations of organic acids (volatile fatty acids; VFA, and branched chain volatile fatty acids; BCVFA) were measured using a gas chromatography system (Varian Chromatography System, model Star 3400; Varian Medical Systems, Palo Alto, CA) and a capillary column (30 × 0.5 mm; Restek Corp., Bellefonte, PA) (Ryan, 1980). 1 mL of 25% metaphosphoric acid was mixed with 5 mL of digesta fluid in a 15 mL centrifuge tube, and the mixture was frozen overnight to maintain the phosphoric acid. After the acidified samples had been frozen, they were neutralised with 0.4 mL of 25% NaOH and vortexed. Following that, 0.65 mL of 0.3 M oxalic acid was added to the samples, and they were vortexed again. After centrifuging the samples for 20 minutes at 3,000 × *g* at 4°C, they were transferred to a gas chromatography vial to be measured.

3.3.10 Gut permeability

The electrophysiological parameters of the cells, such as short-circuit current and transepithelial electrical resistance (TEER), were analysed using revised Ussing chambers (VCC-MC8; Physiologic Instruments Inc., San Diego, CA) comprising pairs of current (Ag wire) and voltage (Ag/AgCl pellet) electrodes built to house in 3% agar bridges and filled with KRB buffer without glucose. 5 mL of KRB buffer solution enriched with 10 $mmol \cdot L^{-1}$ glucose was added to the serosal chambers. Both the mucosal and serosal chambers were constantly gassed with a mixture of 5% carbon dioxide and 5% oxygen (O_2 and CO_2). The chambers' temperature was kept at 37°C by using a water-jacketed reservoir to protect the water from freezing. It was necessary to compensate for any potential discrepancies that may have occurred between the mucosal and serosal chambers prior to tissue mounting. The tissue was placed in Ussing chambers using a tissue slider with a 1 cm^2 opening after the serosal and longitudinal muscle layers were gently peeled away with microforceps. After allowing 10 minutes for the tissue to equilibrate, the short-circuit current and TEER were measured for 10 minutes after the tissue was mounted and dried. Following that, 10 $mmol \cdot L^{-1}$ d-glucose was delivered to the mucosal chamber to examine sodium-dependent glucose

transport, and 10 mmol·L^{-1} mannitol was provided to the serosal chamber to preserve osmotic equilibrium throughout the body (Mrabti et al., 2019). To determine the difference in short-circuit current produced by Na+-glucose cotransporter 1 (SGLT1), we deducted the short-circuit current value before stimulation from the apex after stimulation. After treating the mucosal side with 0.1 mg·mL^{-1} of FITC-D4 (molecular weight 4 kDa; Sigma-Aldrich Co.), a sample (1 mL) was collected from the serosal side to measure intestinal permeability. The subepithelial side of the KRB was sampled and placed to 96-well plates in a volume of 100 mL. The fluorescence was measured at 485 nm and 528 nm at the excitation and emission wavelengths, respectively, using a Bio-Tek PowerWave HT Microplate Scanning Spectrophotometer (BIO-TEK Instruments, Inc.). The concentrations of FITC-D4 in the KRB buffer (ng·mL^{-1}) were calculated using a standard curve from the literature (R^2 = 0.99). The FITC-D4 flux was measured for one hour using a slide with a well surface area of one cm^2 and expressed as μg.cm^{-2}·hr^{-1} ·mL^{-1}. The FITC-D4 flux was measured using a slide with a well surface area of 1 cm^2.

3.3.11 RNA extraction and real-time PCR analysis

Total RNA was obtained from 50 mg of liquid nitrogen pulverised mid-jejunal tissue samples using an RNAqueous total RNA extraction kit, which was designed specifically for this purpose (Ambion Inc.). In this study, the concentration and OD260:OD280 ratio of extracted RNA samples were determined using a Nanodrop UV-Vis spectrophotometer 2000 (Thermo Fisher Scientific Inc.) in Ottawa, Ontario, Canada. The concentration and OD260:OD280 ratios of all extracted RNA samples were between 1.9 and 2.1. The RNA samples were kept at a temperature of -80°C in preparation for further analysis. The first-strand cDNA was synthesised from a total of 1 μg RNA using an iScript cDNA Synthesis Kit (Bio-Rad, Mississauga, ON, Canada) according to the manufacturer's instructions using an iScript complementary DNA Synthesis Kit. Primer-Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to create all of the primers, which are listed in Table 3.2. Integrated DNA Technologies, Inc. produced the primers used in this study (Coralville, IA).

It was carried out utilizing a CFX Connect Real-Time PCR Detection System and a SYBR Green Supermix (Bio-Rad) on a CFX Connect Real-Time PCR Detection System (Bio-Rad; Omonijo et al., 2018b). One 1 μL of cDNA was added to a total volume of 20 μL which included 10 μL of

SYBR Green supermix and 300 nmol· L^{-1} of forward and reverse primers. Each reaction was subjected to the following thermal conditions: 3 minutes at 95°C, followed by 40 cycles of 20 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C. Internal control Cyclophilin-A (CycA), which served to standardize the quantity of RNA utilized in the real-time PCR for all of the samples, was employed as an internal control. A melting curve procedure was carried out to ensure that each PCR product was specific to its target. Normalizing the target mRNA abundance with that of a reference gene of choice was performed, and the relative mRNA abundance was measured by using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001). When the gene is amplified above the threshold of 30 fluorescence units, threshold cycle (Ct) values are acquired. These values are obtained at the cycle number at which the gene is amplified. The efficiencies of real-time PCR were determined by amplification of a dilution series of DNase-treated RNA using the formula 10 (1/slope) for each dilution series (Pfaffl, 2001). All of the primers utilized in this investigation had efficiencies ranging from 96% to 105%, according to the results. Each run was accompanied by negative controls that did not include cDNA, and each sample was examined in triplicate for each of the genes tested.

Table 3.2 Primer sequences for real-time quantitative PCR analysis

Genes[1]	Amplicon	Sequence, 5′ to 3′	References
IL-10	220	CATCCACTTCCCAACCAGCC CTCCCCATCACTCTCTGCCTTC	(Lee & Kang, 2017)
IL-6	151	AAGGTGATGCCACCTCAGAC TCTGCCAGTACCTCCTTGCT	(Deng et al., 2013)
TNF-α	151	ATGGATGGGTGGATGAGAAA TGGAAACTGTTGGGGAGAAG	(Drews et al., 1990)
TLR2	109	ACATGAAGATGATGTGGGCC TAGGAGTCCTGCTCACTGTA	(Tohno et al., 2005)
TLR4	108	GCCATCGCTGCTAACATCATC CTCATACTCAAAGATACACCATCGG	
OCLN	93	CTGTGGATGTCCTGCGTGT GGTTGCTTGCAAAGTGGTGTT	(Lee & Kang, 2017)
ZO1	200	GATCCTGACCCGGTGTCTGA TTGGTGGGTTTGGTGGGTT	(Omonijo et al., 2018)
MUC2	90	CCAGGTCGAGTACATCCTGC GTGCTGACCATGGCCCC	(Janghan et al., 2020)

[1]*IL*-10, interleukin 10; *IL*-6*,* interleukin 6; *TLR*2, toll-like receptor 2; *TLR*4*,* toll-like receptor 4; OCLN, occludin; *ZO*1, Zonula occludens 1; *MUC*2*,* Mucin 2

3.3.12 Microbes quantification by real-time PCR

The genic DNA from the collected colon digesta of weaned piglets was extracted to use the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) by following the instruction of manufacturer and then, DNA was stored at −80° C. After DNA collection, the quantity as well as the quality of the DNA were measured by a Nanodrop UV-Vis spectrophotometer 2000 (Thermo Fisher Scientific Inc., Ottawa, ON, Canada). To quantify microbes in the colon digesta, the real-time PCR method was used as described in ***3.3.11.***

3.3.13 Plasma cytokines

Blood samples of weaned piglets were firstly centrifuged at 2,000 × g at 4°C for 10 min to collect the supernatant plasma into vials. The blood plasma samples were kept in freezer at –80°C for the use of cytokine analysis. The kits of porcine interleukin (*IL*)-10 and *TNF-α* (Porcine *IL*-6 ELISA Kit and Porcine *IL*-10 ELISA Kit; Sigma-Aldrich) were used to measure the quantity of anti-inflammatory cytokine *IL*-10 and Pro-inflammatory cytokine *TNF-α* in plasma samples following the instructions of the manufacturer. The last step was to check the optical densities via a spectrophotometer (SoftMax Pro; Molecular Devices, Abingdon, Oxfordshire, UK) with the emission wavelength of 540 nm.

3.3.14 Intestinal morphology analysis

In order to assess villus height (VH), crypt depth (CD), and the ratio of VH to CD, the Alcian blue/the periodic acid–Schiff (AB/PAS) staining was conducted as published by Koo et al. (2020). After being fixed in 10% neutral-buffered formalin, the samples were embedded in paraffin and sliced into 5-micron pieces before being mounted on glass slides. Dewaxed sections were immersed for 5 minutes in xylene, 95% ethanol, and 100% ethanol, twice in each solution, for a total of 8 cycles. It was required to wash the samples for 15 minutes at room temperature in Alcian blue solution (pH 2.5), following by a 2-minute wash with water, and then to immerse for 10 minutes in Schiff reagent, followed by a 10-minute rinse with water. The samples were then decolorized with hematoxylin for 10 seconds before being cleaned and dehydrated once more. Each sample was observed and photographed using an Axio Scope A1 microscope (Carl Zeiss Micro-Imaging GmbH, Göttingen, Germany) and an Infinity 2 digital camera to evaluate the quantitative amount of AB/PAS staining present (Lumenera Corporation, Ottawa, ON, Canada). The Infinity Analyze programme was used to calculate the VH, CD, and VH:CD ratios (version 6.5.4; Lumenera Corporation, Ottawa, ON, Canada). Each sample which was measured included between 50 and 150 villi and crypts.

3.3.16 Statistical analyses

Statistical analyses were conducted using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) with each individual animal as the experimental unit. The model included experimental diet (NC, CC, AGP, HP1, HP2) as the fixed variable with a completely randomized design. The single degree of freedom orthogonal contrast to test: 1) the effect of ETEC challenge on weaned piglets (NC vs. CC); 2) the effect of AGP on the performance of weaned piglets (CC vs. AGP); 3) the effect of CM NSP HP on ETEC challenged piglets (CC vs. HP1 + HP2); 4) the effect of different concentrations of CM NSP HP (HP1 vs. HP2).

The LSMEANS statement with the Tukey-adjusted PDIFF option was employed to calculate and compare differences among treatment means. Results in tables were shown as least-square means and pooled standard errors of the means, and results in figures shown as mean ± SEM. The comparison was presented as $*0.05 < P$ $**P < 0.01$. Differences were considered significant at $P < 0.05$, and trends ($0.05 \leq P \leq 0.10$) were also presented.

3.4 Results

3.4.1 Hydrolysis products analysis

As shown in Table 3.2 and Table 3.3, the concentrations of crude fat, crude protein and component sugars concentrations of products in CM NSP HP were measured.

Table 3.2 Crude fat and crude protein concentrations of products derived from CM NSP hydrolysis by CE[1,2] (DM basis)

Items	%
Crude Fat	0.63%
Crude Protein (N×6.26)	43.5%
Ash	13.34%
Carbohydrates	13.85%

[1] NDF; Neutral detergent fiber, ADF; Acid detergent fiber
[2] CE, the enzyme blend contained Pectinase A (0.4%), Pectinase B (0.4%), Xylanase B (0.4%)

Table 3.3 Component sugars concentrations of products derived from CM NSP hydrolysis by CE[1]

Component Sugars	mg/g
Rhamnose	4.82
Arabinose	24.87
Xylose	22.02
Mannose	8.64
Galactose	7.95
Glucose	21.27
Uronic acids	49.05
Total	**138.59**

[1] CE, the enzyme blend contained Pectinase A (0.4%), Pectinase B (0.4%), Xylanase B (0.4%)

3.4.2 Growth performance and fecal score

As shown in Table 3.4, during pre-challenge (0-7d), there were no differences in BW, average daily gain (ADG), average daily feed intake (ADFI), G: F among treatments ($P > 0.05$). ETEC F4 infection significantly decreased ($P < 0.05$) the ADG of CC weaned piglets when compared with NC piglets during post-challenge period (7-14 d). The G: F of CC piglets was also decreased compared with NC piglets ($P < 0.05$). Moreover, CM NSP HP significantly increased G: F compared with CC when challenged with ETEC F4. During the whole period (0-14 d), G: F of piglets from HP1 and HP2 were increased compared with those piglets from CC treatment ($P < 0.05$). In addition, the AGP supplementation tended to increase the G: F during pre-challenge and post-challenge period ($P = 0,09$; $P = 0.06$), while G: F of PC piglets fed with AGP was significantly greater ($P < 0.05$) than that of weaned piglets in NC group during the whole period.

As shown in Figure 3.1, inoculation with ETEC F4 induced diarrhea in the CC piglets when compared with NC piglets during the post-challenge period ($P < 0.05$). Except for post-challenge day 2 and day 6, piglets fed HP1 and HP2 had a lower ($P < 0.05$) fecal score compared with the piglets fed CC. The results showed that AGP piglets had a lower diarrhea score ($P < 0.05$) compared with CC piglets during post-challenge period.

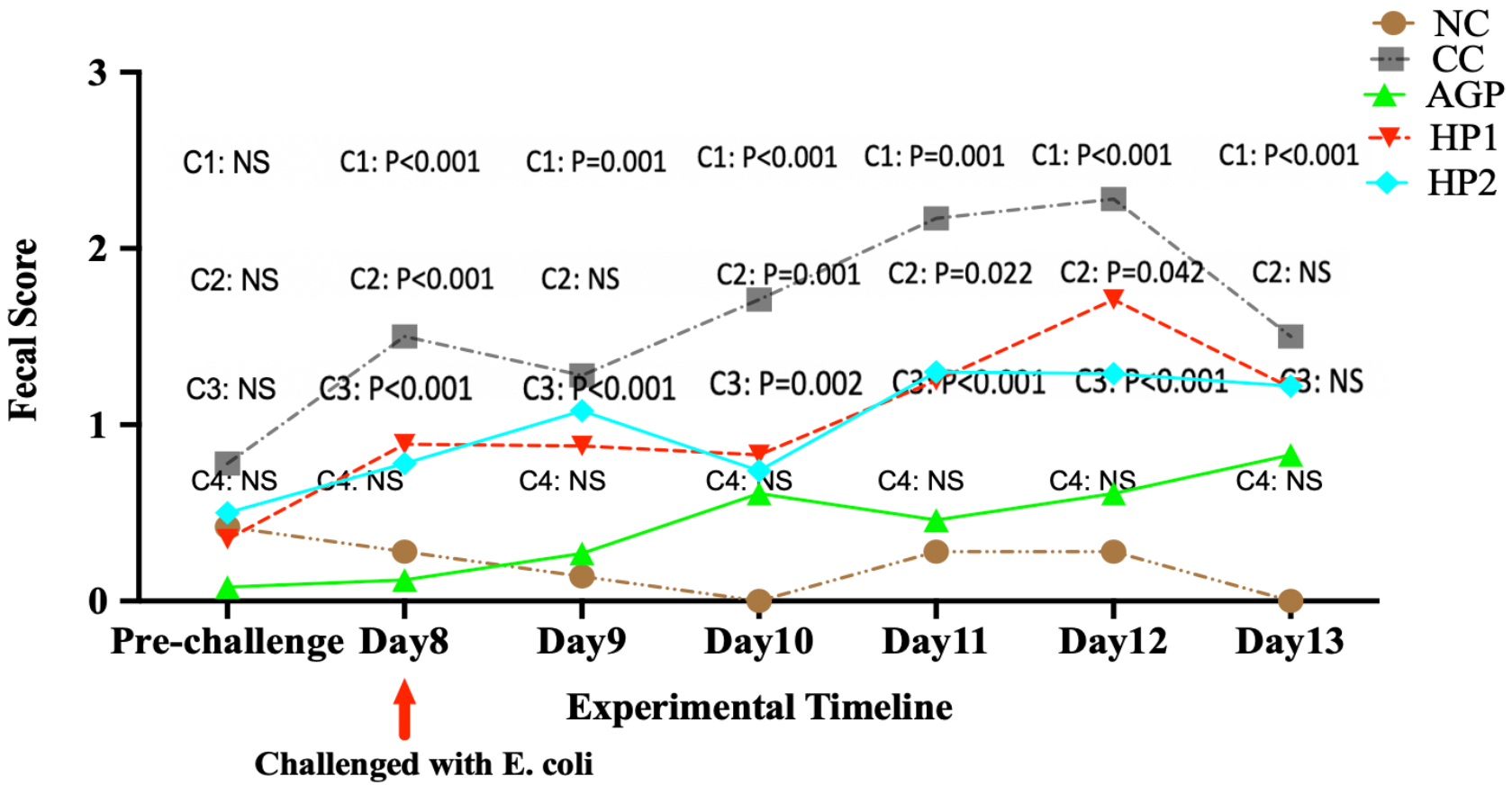
3.4.3 Gut permeability

In Table 3.5, ETEC F4 infection tended to increase the FITC-D4 flux of the CC pigs when compared with the NC pigs ($P = 0.07$). The supplementation of AGP had a lower FITC-D4 flux compared with CC piglets ($P < 0.01$). The inoculation of ETEC F4 decreased ($P < 0.05$) the TEER value of CC pigs when compared with the piglets in NC group. The supplementation of AGP had a higher TEER compared with CC piglets ($P < 0.01$). However, the supplementation of CM NSP HP did not affect FITC-D4 flux and TEER value ($P > 0.10$) and there were no differences between HP1 and HP2 ($P > 0.10$).

Table 3.4 Effect of CM NSP HP on the growth performance of weaned piglets during the pre-challenge period (0 to 7 d),

		Challenged					*P*-value			
Items	NC	CC	AGP	HP1	HP2	SEM	NC vs. CC	CC vs. HP	CC vs. AGP	HP1 vs. HP2
BW (kg)										
Day 0	7.68	8.16	8.05	8.27	8.32	0.349	0.355	0.749	0.842	0.917
Day 7	9.25	9.69	9.98	9.81	9.78	0.522	0.561	0.871	0.713	0.968
Day 14	13.29	12.36	13.50	12.85	12.91	0.797	0.424	0.610	0.347	0.956
ADG (g/day)										
0-7 days	223	218	275	219	208	37.567	0.935	0.917	0.324	0.829
7-14 days	578	381	503	433	446	55.302	0.020	0.406	0.152	0.868
Overall	400	300	389	327	327	42.981	0.116	0.623	0.177	0.990
ADFI (g/day)										
0-7 days	281	262	261	279	256	37.925	0.727	0.996	0.993	0.820
7-14 days	665	665	619	567	535	73.114	0.995	0.223	0.674	0.743
Overall	473	464	440	417	395	50.736	0.899	0.376	0.759	0.748
G: F (g/g)										
0-7 days	0.75	0.85	1.17	0.79	0.81	0.110	0.408	0.566	0.094	0.898
7-14 days	0.88	0.63	0.80	0.75	0.83	0.059	0.006	0.035	0.064	0.358
Overall	0.85	0.68	0.87	0.77	0.82	0.048	0.022	0.060	0.012	0.408

Figure 3.1 Effect of CM NSP HP on the fecal score[1] of weaned piglets during pre-challenge (0-7 d) and post-challenge[2,3]



[1]Fecal score: 0 = well-formed feces; 1 = soft feces; 2 = fluid feces; 3 = liquid feces.
[2]NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC
[3]C1: NC vs. CC, C2: CC vs. AGP, C3: CC vs. HP and C4: HP1 vs. HP2

Table 3.5 Effect of CM NSP HP on electrophysiological properties including TEER[1] and flux of FITC-D4 of weaned piglets' jejunum mounted in Ussing chambers[2]

		Challenged					*P*-value			
Item	NC	CC	AGP	HP1	HP2	SEM	NC vs. CC	CC vs. HP	CC vs. AGP	HP1 vs. HP2
FITC-D4 flux, $ng \cdot cm^{-2} \cdot hr^{-1} \cdot mL^{-1}$	146.64	291.65	57.73	217.07	208.18	55.562	0.074	0.248	0.007	0.907
TEER, $\Omega \cdot cm^2$	108.26	45.07	67.09	54.30	44.34	8.776	0.001	0.691	0.097	0.412

[1]TEER, transepithelial electrical resistance
[2]NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC

3.4.4 Intestinal morphology

As shown in Table 3.6, ETEC F4 inoculation decreased mid-jejunal VH and VH/CD, but increased CD in the NC piglets when compared with the NC piglets ($P < 0.05$). Piglets fed with the diets supplement with AGP had a higher VH than piglets in CC group. Also, CM NSP HP addition tended to improve the VH of HP1 and HP2 pigs ($P = 0.09$) whereas decreased the CD compared with the CC piglets ($P < 0.05$). Both supplementation of AGP and HP increased VH/CD of jejunum compared with NC weaned piglets ($P < 0.05$). However, there were no differences in VH, CD, and VH/CD between HP1 and HP2 pigs ($P > 0.10$).

3.4.5 Microbial activities

In Table 3.7, the AGP supplementation in AGP group showed a lower pH in jejunum digesta compared with the CC group ($P < 0.05$). The CM NSP HP did not affect pH in jejunum digesta and feces of HP pigs compared with CC pigs ($P > 0.10$). The HP supplementation tended to decrease the VFA concentration in jejunum digesta compared with CC group ($P = 0.08$), while AGP had no effect on the VFA ($P > 0.10$). The ETEC F4 inoculation decreased the concentration of BCVFA in jejunum digesta ($P < 0.05$) compared to NC group. There were no differences in VFA concentration in feces, BCVFA concentration in feces, and Ammonia N concentration in jejunum digesta and feces among treatment groups ($P > 0.10$). There were no differences in VFA, BCVFA, and Ammonia N concentrations and pH between HP1 and HP2 groups ($P > 0.10$).

3.4.6 Relative mRNA abundance of genes related to gut barrier function and immune system

To further analyze the inflammatory changes in jejunum, relative gene expressions of inflammatory cytokines *TNF-α*, *IL-10, IL-6, TLR 2* and *TLR 4* were determined. As shown in Figure 3.2, the inoculation of ETEC F4 increased the gene expression of *TLR 2* and *TLR 4* compared to NC group ($P < 0.05$). The PC treatment had a lower gene expression of *IL-10* compared with the NC group ($P < 0.05$). Also, the HP2 had a lower gene expression of *TNF-α*, *IL-6, TLR 2* and *TLR 4* compared to the CC group ($P < 0.05$). The relative mRNA abundance of genes *OCLN, ZO1* and *MUC2* associated with gut barrier function were also determined. The HP1

treatment increased ($P < 0.05$) the *OCLN* expression in the mid-jejunum of pigs but had no effect on the expression of *ZO1* and *MUC2* ($P > 0.10$). The HP2 group showed a higher expression of *OCLN, ZO1* and *MUC2* when compared with the CC group ($P < 0.05$). However, the AGP supplementation did not have effect on the gene expression of *OCLN, ZO1* and *MUC2* ($P > 0.10$) in jejunum tissue.

3.4.7 Microbes abundance in the colon digesta

As shown in Figure 3.3, the HP2 group had a higher DNA abundance of butyrate procuring bacteria (*BCOAF*) and *Lactobacillus* ($P < 0.05$) in the colon digesta than the CC group, while HP1 and AGP group had no difference with the CC group in the DNA abundance of *BCOAF* and *Lactobacillus* ($P > 0.10$). However, the supplementation of AGP and CM NSP HP did not affect the DNA abundance *of Bifidobacterium*, *Clostridium*, *Salmonella* and *E. coli* when compared with the CC group ($P > 0.10$).

3.4.8 Plasma cytokines

As shown in Figure 3.5, the ETEC F4 inoculation leaded to a higher level of *TNF-*α in plasma of weaned pigs compared with NC ($P < 0.05$) in this study. However, there were no differences in the level of plasma *TNF-*α among CC with AGP and HP groups ($P > 0.10$). In addition, the AGP supplementation had a higher level of *IL*-10 compared with CC group ($P < 0.05$) when pigs challenged with ETEC. However, the supplementation of CM NSP HP did not show any difference in the plasma *IL*-10 in piglets challenged with ETEC ($P > 0.10$) compared to CC group.

Table 3.6 Effects of CM NSP HP on morphology including VH, CD and VH/CD in the mid-jejunum of weaned piglets[1]

		Challenged					*P*-Value			
Item	NC	CC	AGP	HP1	HP2	SEM	NC vs. CC	CC vs. AGP	CC vs. HP	HP1 vs. HP2
VH	2.94	1.69	2.34	2.15	2.04	0.979	<0.001	0.031	0.094	0.690
CD	1.50	1.56	1.14	1.31	1.38	0.080	0.578	0.001	0.033	0.573
VH/CD	1.96	1.09	2.06	1.64	1.51	0.123	<0.001	<0.001	0.003	0.449

[1]NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC

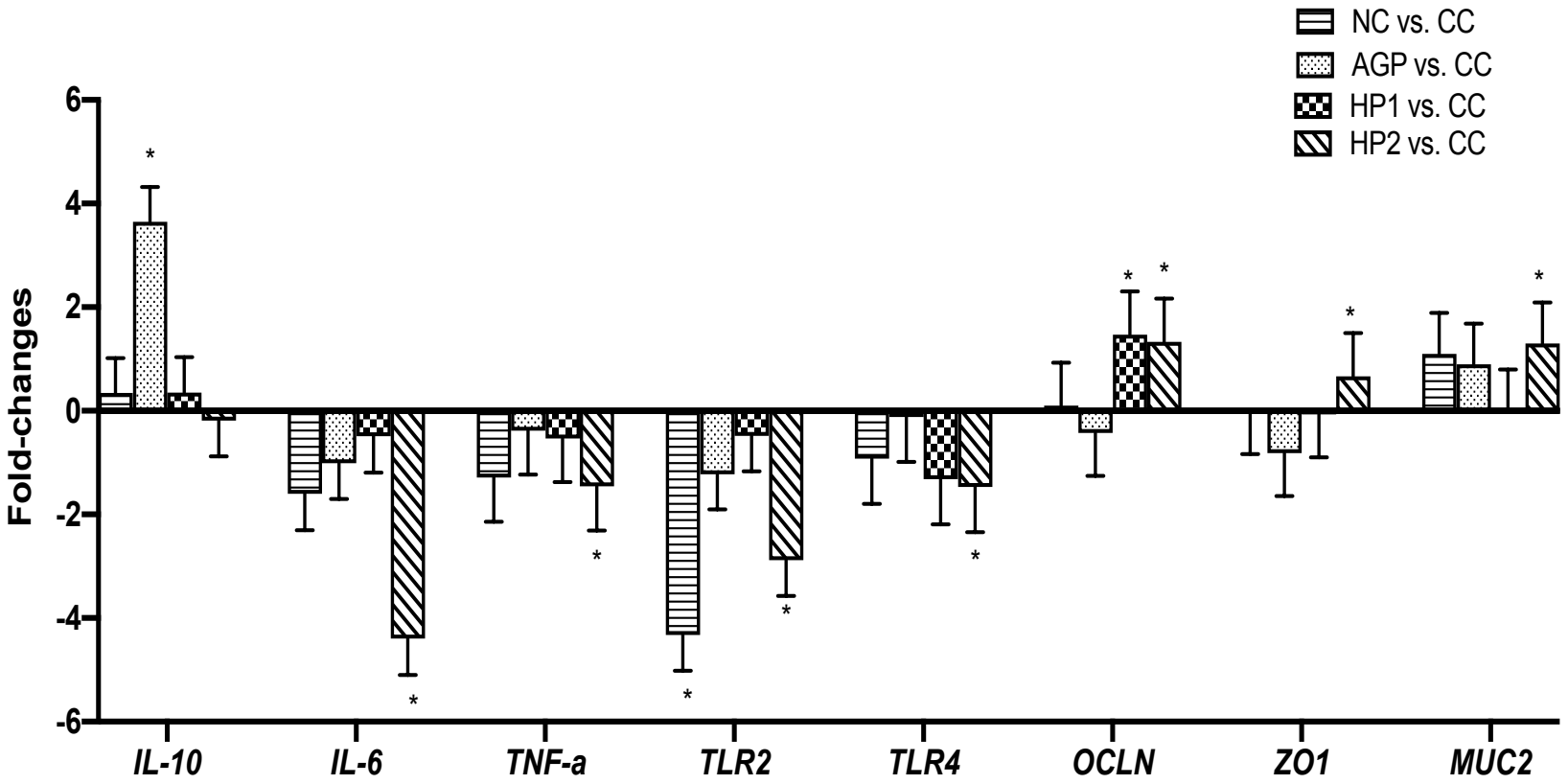
Table 3.7 Effect of CM NSP HP on the VFA, BCVFA and ammonia N of jejunum digesta and feces of weaned piglets

		Challenged					*P*-Value			
Item	NC[1]	CC	AGP	HP1	HP2	SEM	NC vs. CC	CC vs. HP	CC vs. AGP	HP1 vs. HP2
Jejunum										
pH	6.65	6.66	6.34	5.95	5.93	0.186	0.963	0.004	0.254	0.927
VFA[2] (mmol/L)	1.03	0.80	1.33	0.73	0.77	0.200	0.422	0.807	0.082	0.887
BCFA[3] (mmol/L)	0.22	0.14	0.17	0.12	0.14	0.016	0.001	0.787	0.303	0.294
Ammonia N (mg/L)	2.94	2.89	2.70	2.53	2.43	0.238	0.881	0.164	0.574	0.770
Feces										
pH	6.59	6.07	5.97	5.98	5.95	0.153	0.026	0.592	0.682	0.863
VFA (mmol/L)	14.95	13.97	17.11	15.24	15.02	1.834	0.715	0.598	0.245	0.932
BCVFA (mmol/L)	1.36	1.08	1.34	1.39	1.41	0.210	0.359	0.226	0.396	0.952

Ammonia N (mg/L)	6.55	6.75	7.58	7.08	6.35	1.118	0.898	0.982	0.614	0.647

[1]NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC
[2] VFA, volatile fatty acid; sum of acetate, propionate, and butyrate
[3] BCVFA, branched chain volatile fatty acids; sum of isobutyrate, isovalerate and valerate.

Figure 3.2 Effects of CM NSP HP on the relative mRNA abundance of genes associated with gut barrier integrity, nutrient transporters, immune system, and digestive enzymes in the mid-jejunum of weaned piglets.



[1]NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC F4

[2]*$0.05 < P$

Figure 3.3 Effects of CM SNP HP on DNA abundance of microbes in the colon digesta in weaned piglets.

DNA abundance of *Bifidobacterium*, *BCOAF*, *Lactobacillus*, *Clostridium* and *Salmonella* in the colon digesta (20 cm from the ileum–cecum junction) was measured in the NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC F4. Data were presented as log10 (gene copies·DNA^{-1}). Each value represents the mean ± SEM. The comparison was presented as *0.05 < *P*.

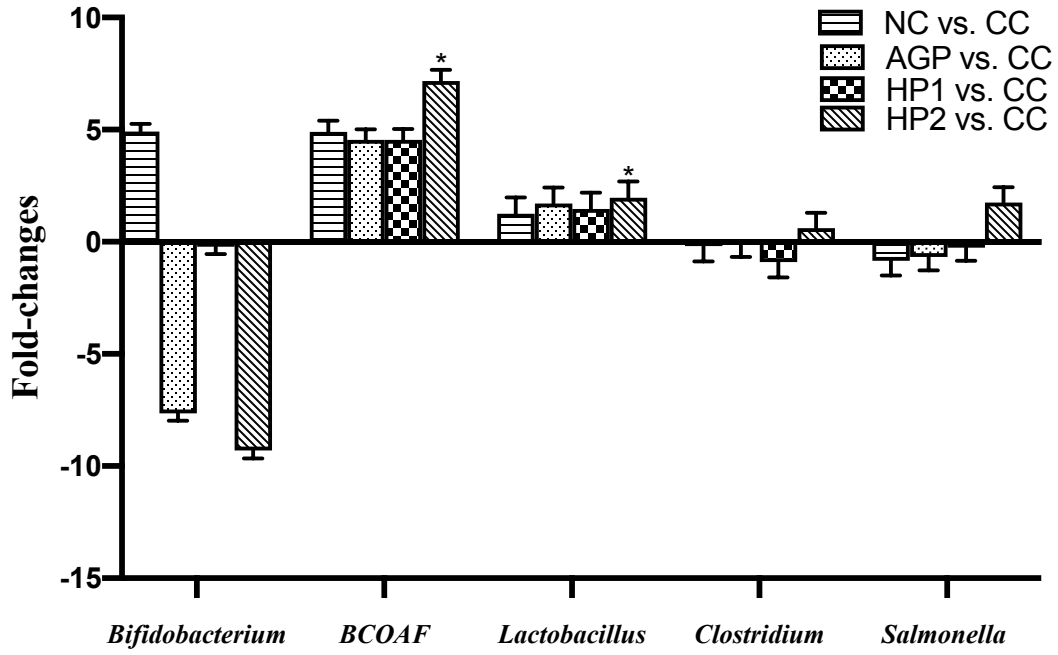
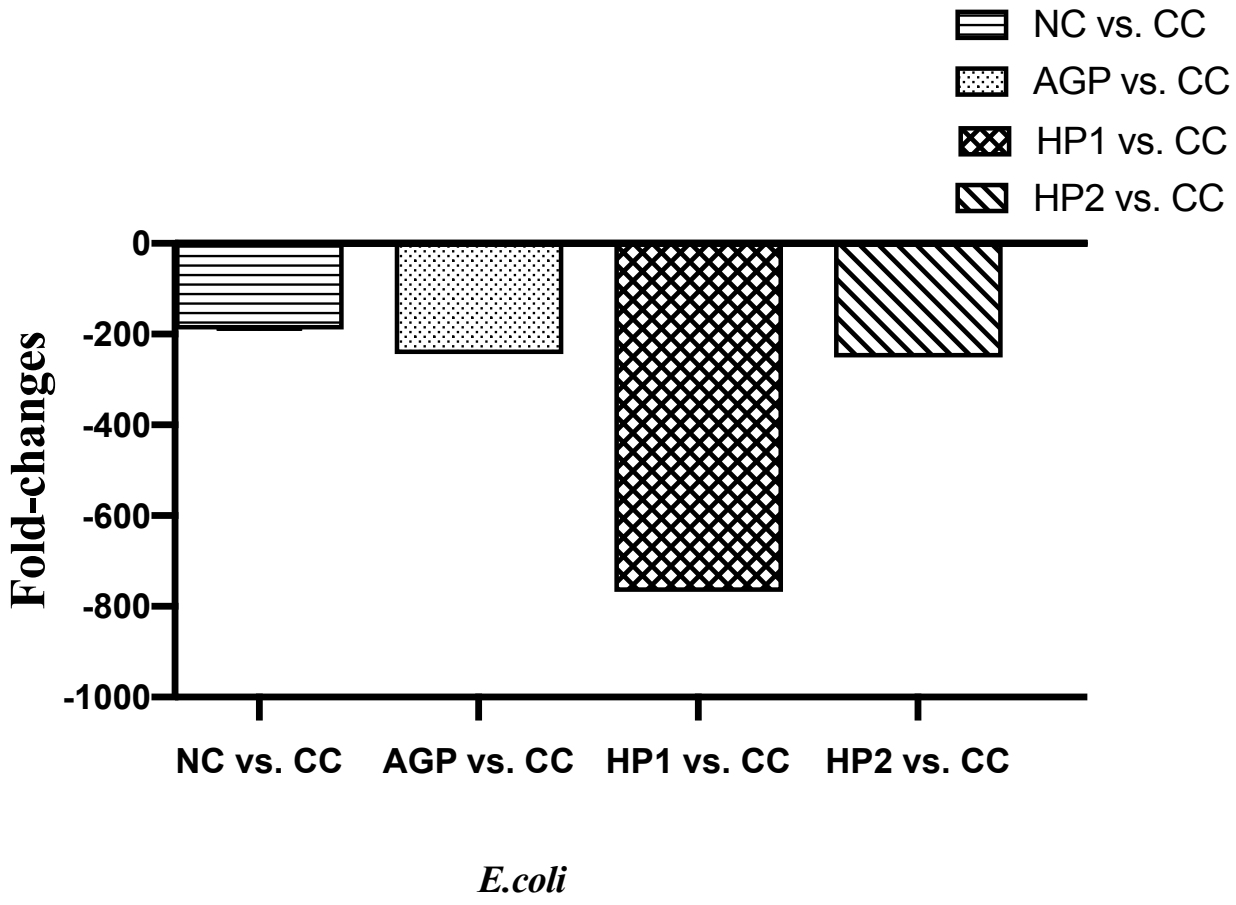
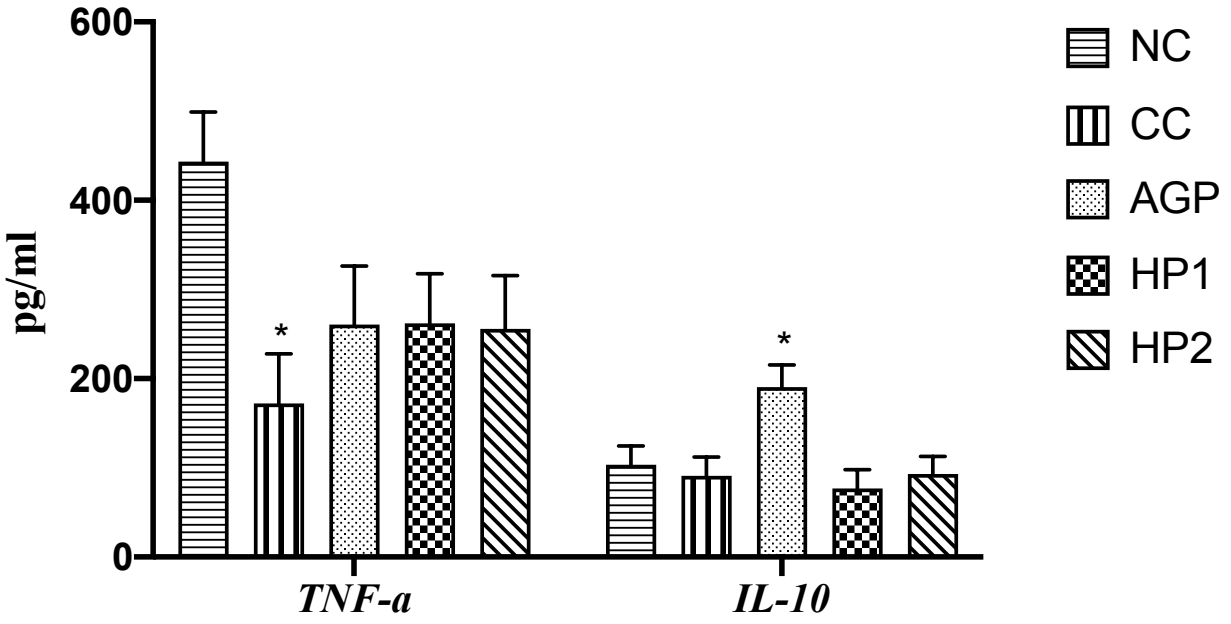


Figure 3.4 Effects of CM SNP HP on DNA abundance of *E. coli* in the colon digesta in weaned piglets.



[1]NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC F4

Figure 3.5 Effect of CM NSP HP on the plasma cytokines of weaned piglets after 24h-chanllenge.



[1]NC, no challenge; CC, challenged control with ETEC F4; AGP, CC+ 0.1% Aureomycin challenged with ETEC F4; HP1, CC + 0.25% CM NSP HP challenged with ETEC F4; HP2, CC + 0.5% CM NSP HP challenged with ETEC F4
[2]*IL*, interleukin; *TNF-α*, tumor necrosis factor alpha

3.5 Discussion

Our previous study found NSP HP helped keep fluid balance of small-intestine segments from weaned piglets challenged with ETEC. Besides, Kiarie et al. (2010) found that the supplementation of 0.05% NSP HP from wheat, CM and SBM decreased the ileal adherent ETEC counts, digesta ammonia N concentration and caused less diarrhea of weaned piglets challenged with ETEC. This was because some xylo-oligomers, mano-oligomers, gluco-oligomers in NSP HP have similar effects with antibiotic by interfering with attachment to the enterocytes and modulating the composition of beneficial bacteria in piglets. Thus, in the present study, we continued to study the antibiotic effect of NSP HP from CM in weaned pigs.

During the weaning period, pigs encounter many stresses from the environment and diet changes and have high possibility to get infected with PWD. Thus, it is essential to find efficient method to improve the gut health and growth of weaned piglets, and replace the use of AGP. And *E. coli* has been considered as the most important pathogens for piglets suffering PWD (Pluske et al., 2018). Therefore, we used the ETEC model to challenge the weaned piglets with 5 ml (5×10^6 CFU/mL). The results showed 7.8% of mortality in unchallenged group and challenged groups (3 dead pigs out of 38 pigs). Based on the results of fecal score and body temperature between NC and other treatments challenged with ETEC, the symptoms of ETEC F4 infection caused by ETEC F4 toxins were successfully achieved.

In the present study, during post-challenge period, CM NSP HP increased G: F that could be partly explained by the reduced diarrhea and increased morphology. The potential mechanisms related to decreased performance of those piglets challenged with ETEC may include decreased efficiency of nutrient digestion or absorption; inflammation factors; increased diarrhea, which cause loss of water in the body and decreased available nutrients for pigs because ETEC F4 may have competed for nutrients with the host (Heo et al., 2013). These results are in agreement with previous research, showing an improvement of ADFI and feed efficiency of weaned pigs fed oligosaccharide-based polymer infected with ETEC (Kim et al., 2021). In the study of Kiarie (2010), NSP HP were shown to benefit ADG in pigs. Besides, in the current study, the results showed that the supplementation of AGP also increased the GF ratio of ETEC challenged weaned piglets during the whole period. Thus, both CM NSP HP and AGP mitigated decreased growth performance due to ETEC F4 infection.

The pathogenesis of ETEC F4 mainly relies on two factors: ETEC F4 virulence and F4 fimbriae receptors (Kim et al., 2019). Fimbriae support ETEC to adhere and colonize in the small intestine, then the colonized *E. coli* release enterotoxins, including heat-labile toxins, heat-stable toxins, or shiga toxins. Heat-labile toxins are closely related to increased gut permeability by increasing chloride and water secretion and decreasing sodium and chloride absorption. In addition, heat-stable toxin b could lead to the histological damage in pigs, whereas shiga toxins are highly involved in inflammation and increased immune cytokines (Kim et al., 2019; Tarr et al., 2005). In

our study, the supplementation of CM NSP HP and AGP reduced the fecal diarrhea in ETEC piglets. The reduced gut permeability was observed in ETEC challenged piglets fed with AGP. These findings are in agreement with previous study (Kiarie et al., 2010), which indicated that at 48 h post-challenge of ETEC, pigs fed diet containing combination of NSP HP and EYA showed low incidence of diarrhea. The potential reason is likely due to the improved gut health in pigs fed CM NSP HP and AGP, as indicated by the increased mRNA expression of *ZO-1*, *OCLN* and *MUC2* in jejunal mucosa of pigs with addition of CM NSP HP and AGP. Mucins and tight junction proteins are the main factors in the formation of gut barrier. *MUC2* which is a main core polypeptide of mucins related with luminal contents plays a major role in defense against inflammation (Kim et al., 2019).

Previous study has indicated that ETEC F4 toxins could reduce the mRNA expression of *MUC2* in ileal mucosa of weaned pigs (Liu et al., 2013). The peripheral membrane protein *ZO1* and *OCLN* are major factors of tight junction. It has been showed that tight junction is highly related with the gut permeability (Bruewer et al., 2003). In our results, the reduced gut permeability was observed in ETEC challenged piglets fed with AGP. Besides, enterocytes are related to nutrient digestion and absorption because brush border digestive enzymes and nutrient transporters are expressed in the enterocytes, and therefore, the VH and CD are correlated with surface area for nutrients digestion and absorption capacity (Kong et al., 2018). In this study, ETEC caused a decreased VH and higher CD, which is consistent with the damaged intestinal barrier by ETEC F4 toxins. Most cells in the crypt have secretory capacity and cells in villous have absorption function in jejunum, thereby an increase in VH:CD results in the increased net absorption (Park et al., 2020; Woyengo et al., 2011). Therefore, the increased jejunal VH:CD by feeding AGP and CM NSP HP to ETEC infected pigs could be explained by the damaged gut barrier and the reduction in the frequency of diarrhea. Overall, CM NSP HP prevented the damage from ETEC F4 infections and protected the intestinal permeability and morphology which were similar to the effect of AGP on weaned pigs during the post-challenge period.

The profile of microbiota could be changed in weaned pigs when challenged with *E.* coli. In order to determine the relative abundance of microbes, PCR assay analysis also was performed in our study. Pigs fed diets containing CM NSP HP and AGP showed lower ETEC numbers which

coincided with less severity of the ETEC-infection associated symptoms as shown by lower visual assessment of fecal consistency. This observation was also shown in our previous study (Kiarie et al., 2010) in which there was a decreased relative abundance of ETEC in the ileal mucosa of pigs fed with NSP HP. An increased abundance of *Lactobacillus* populations was observed in pigs with CM NSP HP during ETEC infection. *Lactobacillus* strains are known to exhibit attributes related to immune modulation, colonization resistance, intestinal barrier homeostasis, and pathogen antagonism (Zhang et al., 2015). This is in agreement with results of another study, in which NSP HP supported higher *Lactobacillus* numbers in ETEC challenged pigs (Kiarie et al., 2010). The gut microbiota provides essential capacities for the fermentation of indigestible carbohydrates into SCFA. Pigs fed AGP appeared to present an improved fermentation capacity, as indicated by the greater VFA concentration in jejunum digesta. The potential reason is that VFA, especially acetate and butyrate, have beneficial effect in improving gut barrier function and protecting the host against bacterial infections (Fukuda et al., 2011; Luying et al., 2009). Moreover, decreased pH by CM NSP HP in jejunum digesta was found in our study, which may have been meditated by the improved fermentation in the intestine using CM NSP HP as substrates. In agreement with our previous study (Kiarie et al., 2010), ETEC infected pigs fed diets containing NSP HP showed lower gastric pH. Low pH in digesta could benefit pig gut health as low pH has been shown to inhibit the growth of pathogenic bacteria (Suiryanrayna & Ramana, 2016), these observations suggest that CM NSP HP supported gut health of pigs when challenged with ETEC.

TNF-α, the typical pro-inflammatory cytokine and *IL-10*, the anti- inflammatory cytokine and have been used as potential bio markers for bacterial infections in piglets (Li et al., 2009). Canola meal NSP HP did not change the plasma cytokine levels at 24h post-challenge in the current study. We speculated that the potential reason is the collection time of blood samples since pigs showed severe diarrhea after 2 days post challenge. In a previous study, pigs fed diets with AGP or NSP HP showed changes in serum pro-inflammatory cytokines at 48h post-challenge (Kiarie et al., 2010). To further analyze the inflammatory changes, the real time PCR analysis demonstrated that 0.5% of CM NSP HP addition decreased pro-inflammatory cytokines *IL-6*, *TNF-α*, *TLR2* and *TLR4* whereas the supplementation of AGP increased pro-inflammatory cytokines the *IL-10*. This might be attributed to the CM NSP HP against pathogens in the gut by interfering with attachment to the enterocytes and increasing the abundance of *Lactobacillus*.

3.6 Conclusions

In conclusion, ETEC F4 infection decreased growth performance, influenced the balance of microbes, induced inflammatory response and diarrhea, damaged gut morphology, and decreased the intestinal permeability of weaned piglets. However, the supplementation of CM NSP HP and AGP increased feed efficiency and decreased the incidence of diarrhea in weaned piglets challenged with ETEC F4. Canola meal NSP HP protected gut barrier integrity from ETEC F4 infection. The supplementation of CM NSP HP and AGP enhanced intestinal morphology in weaned piglets. Therefore, piglets fed diets with CM HP exhibited less severe ETEC-enteritis and had similar effects with AGP. The main functional component of CM NSP HP was assumed to be short-chain ooligosaccharides and peptides, but the mode of action of these products needs to be further studied.

4.0 GENERAL DISCUSSION

Weaning time is an important period in pigs' life since piglets likely experience anorexia caused by many different stressors (e.g., nutritional, psychological, and environmental; Pluske et al., 1997). As well, gut microbiota and immune system could be influenced during the weaning period. Weaning anorexia and under-nutrition are associated with GIT disturbances characterized by reduced digestive and absorptive capacity and enteric diseases. ETEC is the most common strain and PWD in the GIT and could lead to 50% of pig death per year (Heo et al., 2013). Thus, piglets in our experiment were challenged by ETEC model. Indeed, based on the results of the experiment, growth performance, fecal score, microbiota, plasma cytokines and immune system of weaned piglets were influenced by ETEC F4.

It is well known that AGP has been widely used in pig industry for reduction in pathogenic bacteria and improvement in growth and gut health. The supplementation of AGP increased feed efficiency and reduced the fecal diarrhea. Also, AGP was shown to improve the intestinal morphology and gut barrier integrity of piglets when challenged with ETEC in the manuscript. However, the use of AGPs has led to bacterial resistance in animals and human, therefore, many regions has banned or limited the use of AGPs. Thus, many researchers have studied the effective replacements for AGP.

Those alternatives including essential oils, organic acids, probiotic, oligosaccharides, β-glucans and prebiotics. Prebiotics are non-digestible carbohydrates considered as fermented dietary ingredients and defined as any type of food ingredient that has effect on the beneficial GIT microbiota. (Ferreira-Lazarte et al., 2021; Hutkins et al., 2015). Based on our previous study, the HP from wheat meal and flaxseed meal with enzyme digested influenced net absorption of fluid and electrolytes (Kiarie et al.,2010). Compared with wheat meal, CM has higher NSP and been widely used in pig diet due to the lower cost and good nutritive values. CM NSP can be digested by CE and NSP HP could be released. Such products include low molecular weight polysaccharides, oligosaccharides are poorly absorbed and utilized that it may be beneficial for them to pass into the hind gut as oligosaccharides and be fermented by the microflora. CM NSP

HP may be fermented by the microbiota in the large intestine. Thus, CM NSP HP was studied and considered as the alternatives to AGP in weaned pigs' diet in this experiment.
The results indeed showed that CM NSP HP protected gut barrier integrity and immune system from ETEC F4 infection. Also, feed efficiency of piglets and beneficial bacteria was increased in the CM NSP HP treatment. Therefore, piglets fed diets with CM HP exhibited less severe ETEC-enteritis and had similar effects with AGP.

The main functional component in CM NSP HP was assumed to be short-chain oligomers based on pervious study. However, high level of N was analysed in CM NSP HP. This is because peptides could be released by hydrolysis of protein from diet treated with enzymes (Abdel-Haid et al., 2017). Peptides can benefit the performance, nutrient digestibility, intestinal morphology, and microbiota in pigs, thereby are considered as a potential alternative to AGP (Hao et al., 2015). Therefore, we speculated the beneficial effect of CM NSP HP in challenged weaned piglets might attribute to the low-molecular weight polysaccharides, oligosaccharides in the hydrolysis products. However, the mode of action of product still needs to be further studied.

5.0 CONCLUSIONS AND FUTURE STUDIES

CONCLUSIONS

The following conclusions can be drawn from the present research:

1. ETEC F4 infection decreased growth performance, influenced the balance of microbes, induced inflammatory response and diarrhea, damaged gut morphology, decreased the intestinal permeability of weaned piglets.
2. The supplementation of AGP in weaned pigs' diet increased feed efficiency and decreased diarrhea incidence in weaned piglets challenged with ETEC F4. AGP also enhanced intestinal morphology in weaned piglets challenged with ETEC.
3. CM NSP HP protected gut barrier integrity from ETEC F4 infection, increased G:F and reduced the fecal score of weaned pigs.
4. Piglets fed diets with CM HP exhibited less severe ETEC-enteritis and had similar effects with AGP.

FUTURE STUDIES

In the present research, CM NSP HP showed beneficial effect on growth performance, immune system and gut health of weaned pigs. However, 0.25% of CM NSP HP and 0.5% of CM NSP HP did not show any different effect in this study. In consideration of the cost, the optimum amount of CM NSP HP should be further studied. Also, in this study, the main function components of CM NSP HP might be short-chain oligomers, but the mode of action of CM NSP HP was not clear.

Thus, further studies are required to:

1. Investigate the optimum amount of CM NSP HP in weaned pigs diets not only to replace the use of AGP but also to save the cost of producing CM NSP HP.

2. Study and explore the mode of action of CM NSP HP.

6.0 REFERENCES

Aarestrup, F. M. (2015). The livestock reservoir for antimicrobial resistance: a personal view on changing patterns of risks, effects of interventions and the way forward. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1670), 20140085-20140085. https://doi.org/10.1098/rstb.2014.0085

Abdel-Hamid, M., Otte, J., De Gobba, C., Osman, A., & Hamad, E. (2017). Angiotensin I-converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. *International Dairy Journal*, *66*, 91-98. https://doi.org/10.1016/j.idairyj.2016.11.006

Adewole, D. I., Kim, I. H., & Nyachoti, C. M. (2016). Gut health of pigs: challenge models and response criteria with a critical analysis of the effectiveness of selected feed additives - a review. *Asian-Australasian Journal of Animal Sciences*, *29*(7), 909-924. https://doi.org/10.5713/ajas.15.0795

Adewole, D. I., Rogiewicz, A., Dyck, B., & Slominski, B. A. (2016). Chemical and nutritive characteristics of canola meal from Canadian processing facilities. *Animal Feed Science and Technology*, 222, 17-30. https://doi.org/10.1016/j.anifeedsci.2016.09.012

Adhikari, P. A., & Kim, W. K. (2017). Overview of prebiotics and probiotics: focus on performance, gut health and immunity – a review. *Annals of Animal Science*, *17*(4), 949-966. https://doi.org/10.1515/aoas-2016-0092

Bach Knudsen, K. E. (2001). The nutritional significance of "dietary fibre" analysis. *Animal Feed Science and Technology*, *90*(1), 3-20. https://doi.org/10.1016/S0377-8401(01)00193-6

Bailey, M., Haverson, K., Inman, C., Harris, C., Jones, P., Corfield, G., Miller, B., & Stokes, C. (2005). The development of the mucosal immune system pre- and post-weaning: balancing regulatory and effector function. *Proceedings of the Nutrition Society*, *64*(4), 451-457. https://doi.org/10.1079/PNS2005452

Bajaj, B. K., Claes, I. J. J., & Lebeer, S. (2015). Functional mechanisms of probiotics. *Journal of Microbiology, Biotechnology and Food Sciences*, *4*(4), 321-327. https://doi.org/10.15414/jmbfs.2015.4.4.321-327

Bäumler, A. J., & Sperandio, V. (2016). Interactions between the microbiota and pathogenic bacteria in the gut. *Nature, 535*(7610), 85-93. https://doi.org/10.1038/nature18849

Bhandari, S. K., Xu, B., Nyachoti, C. M., Giesting, D. W., & Krause, D. O. (2008). Evaluation of alternatives to antibiotics using an Escherichia coli K88+ model of piglet diarrhea: effects on gut microbial ecology. *Journal of Animal Science*, *86*(4), 836-847. https://doi.org/10.2527/jas.2006-822

Bian, G., Ma, S., Zhu, Z., Su, Y., Zoetendal, E. G., Mackie, R., Liu, J., Mu, C., Huang, R., Smidt, H., & Zhu, W. (2016). Age, introduction of solid feed and weaning are more important determinants of gut bacterial succession in piglets than breed and nursing mother as revealed by a reciprocal cross-fostering model. *Environmental Microbiology*, *18*(5), 1566-1577. https://doi.org/10.1111/1462-2920.13272

Biswas, A., Mohan, N., Dev, K., Mir, N. A., & Tiwari, A. K. (2021). Effect of dietary mannan oligosaccharides and fructo-oligosaccharides on physico-chemical indices, antioxidant and oxidative stability of broiler chicken meat. *Scientific Reports*, *11*(1), 20567-20567. https://doi.org/10.1038/s41598-021-99620-2

Bogere, P., Choi, Y. J., & Heo, J. (2019). Probiotics as alternatives to antibiotics in treating post-weaning diarrhoea in pigs. *South African Journal of Animal Science*, *49*(3), 403-416. https://doi.org/10.4314/sajas.v49i3.1

Boirivant, M., & Strober, W. (2007). The mechanism of action of probiotics. *Current Opinion in Gastroenterology*, *23*(6), 679-692. https://doi.org/10.1097/MOG.0b013e3282f0cffc

Boyer, P. E., D'Costa, S., Edwards, L. L., Milloway, M., Susick, E., Borst, L. B., Thakur, S., Campbell, J. M., Crenshaw, J. D., Polo, J., & Moeser, A. J. (2015). Early-life dietary spray-dried plasma influences immunological and intestinal injury responses to later-life Salmonella typhimurium challenge. *British Journal of Nutrition*, *113*(5), 783-793. https://doi.org/10.1017/S000711451400422X

Brown, D. C., Maxwell, C. V., Erf, G. F., Davis, M. E., Singh, S., & Johnson, Z. B. (2006). The influence of different management systems and age on intestinal morphology, immune cell numbers and mucin production from goblet cells in post-weaning pigs. *Veterinary Immunology and Immunopathology*, *111*(3), 187-198. https://doi.org/10.1016/j.vetimm.2005.12.006

Brown, K., Uwiera, R. R. E., Kalmokoff, M. L., Brooks, S. P. J., & Inglis, G. D. (2016). Antimicrobial growth promoter use in livestock: a requirement to understand their modes

of action to develop effective alternatives. *International Journal of Antimicrobial Agents*, *49*(1), 12-24. https://doi.org/10.1016/j.ijantimicag.2016.08.006

Brown, M. (2011). Modes of action of probiotics: recent developments. *Journal of Animal and Veterinary Advances, 10*(14), 1895-1900. https://doi.org/10.3923/javaa.2011.1895.1900

Bruewer, M., Luegering, A., Kucharzik, T., Parkos, C. A., Madara, J. L., Hopkins, A. M., & Nusrat, A. (2003). Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *The Journal of Immunology (1950)*, *171*(11), 6164-6172. https://doi.org/10.4049/jimmunol.171.11.6164

Buffie, C. G., & Pamer, E. G. (2013). Microbiota-mediated colonization resistance against intestinal pathogens. *Nature Reviews Immunology*, *13*(11), 790-801. https://doi.org/10.1038/nri3535

Casas, G. A., Blavi, L., Cross, T.-W. L., Lee, A. H., Swanson, K. S., & Stein, H. H. (2020). Inclusion of the direct-fed microbial Clostridium butyricum in diets for weanling pigs increases growth performance and tends to increase villus height and crypt depth, but does not change intestinal microbial abundance. *Journal of Animal Science*, *98*(1), 1-12. https://doi.org/10.1093/jas/skz372

Castillo, S., & Gatlin, D. M. (2015). Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: a review. *Aquaculture*, *435*, 286-292. https://doi.org/10.1016/j.aquaculture.2014.10.011

Cervantes-Pahm, S. K., & Stein, H. H. (2010). Ileal digestibility of amino acids in conventional, fermented, and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed to weanling pigs. *Journal of Animal Science*, *88*(8), 2674-2683. https://doi.org/10.2527/jas.2009-2677

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., & Yuan, Z. (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Frontiers in Microbiology*, *5*, 217-217. https://doi.org/10.3389/fmicb.2014.00217

Cho, J. H., Zhao, P. Y., & Kim, I. H. (2011). Probiotics as a dietary additive for pigs: a review. *Journal of Animal and Veterinary Advances, 10*(16), 2127-2134. https://doi.org/10.3923/javaa.2011.2127.2134

Choi, J., Wang, L., Liu, S., Lu, P., Zhao, X., Liu, H., Lahaya, L., Santin, E., Liu, S., Nyachoti, M., & Yang, C. (2020). Effects of a microencapsulated formula of organic acids and essential

oils on nutrient absorption, immunity, gut barrier function, and abundance of enterotoxigenic Escherichia coli F4 in weaned piglets challenged with *E. coli* F4. *Journal of Animal Science, 98*(9), skaa 259. https://doi.org/10.1093/jas/skaa259.

Dadi, T. H., Vahjen, W., Zentek, J., Melzig, M. F., Granica, S., & Piwowarski, J. P. (2020). Lythrum salicaria L. herb and gut microbiota of healthy post-weaning piglets. Focus on prebiotic properties and formation of postbiotic metabolites in ex vivo cultures. *Journal of Ethnopharmacology*, *261*, 113073. https://doi.org/10.1016/j.jep.2020.113073

Daudelin, J.-F., Lessard, M., Beaudoin, F., Nadeau, E., Bissonnette, N., Boutin, Y., Brousseau, J.-P., Lauzon, K., & Fairbrother, J. M. (2011). Administration of probiotics influences F4 (K88)-positive enterotoxigenic Escherichia coli attachment and intestinal cytokine expression in weaned pigs. *Veterinary Research, 42*(1), 69-69. https://doi.org/10.1186/1297-9716-42-69

Deng, J., Li, Y., Zhang, J., & Yang, Q. (2013). Co-administration of Bacillus subtilis RJGP16 and Lactobacillus salivarius B1 strongly enhances the intestinal mucosal immunity of piglets. *Research in Veterinary Science*, *94*(1), 62-68. https://doi.org/10.1016/j.rvsc.2012.07.025

Do, J., Zafar, H., & Saier, M. H. (2017). Comparative genomics of transport proteins in probiotic and pathogenic Escherichia coli and Salmonella enterica strains. *Microbial Pathogenesis*, *107*, 106-115. https://doi.org/10.1016/j.micpath.2017.03.022

Dowarah, R., Verma, A. K., Agarwal, N., Singh, P., & Singh, B. R. (2018). Selection and characterization of probiotic lactic acid bacteria and its impact on growth, nutrient digestibility, health and antioxidant status in weaned piglets. *PloS One*, *13*(3), e0192978-e0192978. https://doi.org/10.1371/journal.pone.0192978

Drews, R. T., Coffee, B. W., Prestwood, A. K., & McGraw, R. A. (1990). Gene sequence of porcine tumor necrosis factor alpha. *Nucleic Acids Research*, *18*(18), 5564-5564. https://doi.org/10.1093/nar/18.18.5564

Dubeski, P. L. (1997). The living gut: an introduction to micro-organisms in nutrition. Animal Feed Science and Technology, 1(68), 190-92. https://doi.org/10.1016/S0377-8401(97)00019-9.

Everaert, N., Van Cruchten, S., Weström, B., Bailey, M., Van Ginneken, C., Thymann, T., & Pieper, R. (2017). A review on early gut maturation and colonization in pigs, including

biological and dietary factors affecting gut homeostasis. *Animal Feed Science and Technology*, *233*, 89-103. https://doi.org/10.1016/j.anifeedsci.2017.06.011

Fairbrother, J. M., Nadeau, É., & Gyles, C. L. (2005). Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews*, *6*(1), 17-39. https://doi.org/10.1079/AHR2005105

Ferreira-Lazarte, A., Moreno, F. J., & Villamiel, M. (2021). Bringing the digestibility of prebiotics into focus: update of carbohydrate digestion models. *Critical Ceviews in Food science and Nutrition*, *61*(19), 3267-3278. https://doi.org/10.1080/10408398.2020.1798344

Fouhse, J. M., Dawson, K., Graugnard, D., Dyck, M., & Willing, B. P. (2019). Dietary supplementation of weaned piglets with a yeast-derived mannan-rich fraction modulates cecal microbial profiles, jejunal morphology and gene expression. *Animal, 13*(8), 1591-1598. https://doi.org/10.1017/S1751731118003361

Fouhse, J. M., Zijlstra, R. T., & Willing, B. P. (2016). The role of gut microbiota in the health and disease of pigs. *Animal Frontiers*, *6*(3), 30-36. https://doi.org/10.2527/af.2016-0031

Fukuda, S., Toh, H., Taylor, T. D., Itoh, K., Kikuchi, J., Morita, H., Hattori, M., Ohno, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J. M., Topping, D. L., & Suzuki, T. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*, *469*(7331), 543-547. https://doi.org/10.1038/nature09646

Gao, J., Yin, J., Xu, K., Li, T., & Yin, Y. (2019). What is the impact of diet on nutritional diarrhea associated with gut microbiota in weaning piglets: a system review. *BioMed Research International*, *2019*, 6916189-6916114. https://doi.org/10.1155/2019/6916189

Garcia, A., Sutherland, M., Pirner, G., Picinin, G., May, M., Backus, B., & McGlone, J. (2016). Impact of providing feed and/or water on performance, physiology, and behavior of weaned pigs during a 32-h transport. *Animals, 6*(5), 31. https://doi.org/10.3390/ani6050031

González-Ortiz, G., Pérez, J., Hermes, R., Molist, F., Jiménez-Díaz, R., & Martín-Orúe, S. (2014). Screening the ability of natural feed ingredients to interfere with the adherence of enterotoxigenic Escherichia coli (ETEC) K88 to the porcine intestinal mucus. British Journal of Nutrition, 111(4), 633-642. doi:10.1017/S0007114513003024

Gresse, R., Chaucheyras-Durand, F., Fleury, M. A., Van de Wiele, T., Forano, E., & Blanquet-Diot, S. (2017). Gut microbiota dysbiosis in postweaning piglets: understanding the keys

to health. *Trends in Microbiology, 25*(10), 851-873. https://doi.org/10.1016/j.tim.2017.05.004

Guerra, N. P., Bernárdez, P. F., Méndez, J., Cachaldora, P., & Pastrana Castro, L. (2007). Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. *Animal Feed Science and Technology*, *134*(1), 89-107. https://doi.org/10.1016/j.anifeedsci.2006.05.010

Hajati, H., & Rezaei, M. (2010). The application of prebiotics in poultry production. *International Journal of Poultry Science*, *9*(3), 298-304. https://doi.org/10.3923/ijps.2010.298.304

Hamer, H. M. H., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R. J. M. (2008). Review article: the role of butyrate on colonic function. *Alimentary Pharmacology & Therapeutics*, *27*(2), 104-119. https://doi.org/10.1111/j.1365-2036.2007.03562.x

He, X., Yu, B., He, J., Huang, Z., Mao, X., Zheng, P., Luo, Y., Luo, J., Wang, Q., Wang, H., Yu, J., & Chen, D. (2020). Effects of xylanase on growth performance, nutrients digestibility and intestinal health in weaned piglets. *Livestock Science*, *233*, 103940. https://doi.org/10.1016/j.livsci.2020.103940

Heo, J. M., Opapeju, F. O., Pluske, J. R., Kim, J. C., Hampson, D. J., & Nyachoti, C. M. (2013). Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. *Journal of Animal Physiology and Animal Nutrition*, *97*(2), 207-237. https://doi.org/10.1111/j.1439-0396.2012.01284.x

Hong, J., Ndou, S. P., Adams, S., Scaria, J., & Woyengo, T. A. (2020). Canola meal in nursery pig diets: growth performance and gut health. *Journal of Animal Science*, *98*(11), 1-18. https://doi.org/10.1093/jas/skaa338

Hopwood, D., & Hampson, D. (2003). Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate post-weaning period. *Weaning the Pig: Concepts and Consequences*, 199-217.

Hou, C., Zeng, X., Yang, F., Liu, H., & Qiao, S. (2016). Study and use of the probiotic Lactobacillus reuteri in pigs： a review. *Journal of Animal Science and Biotechnology*, *7*(1), 26-33. https://doi.org/10.1186/s40104-015-0014-3

Huyghebaert, G., Ducatelle, R., & Immerseel, F. V. (2011). An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal (1997)*, *187*(2), 182-188. https://doi.org/10.1016/j.tvjl.2010.03.003

Isaacson, R., & Kim, H. B. (2012). The intestinal microbiome of the pig. *Animal Health Research Reviews*, *13*(1), 100-109. https://doi.org/10.1017/S1466252312000084

Janghan, C., Lucy, W., Shangxi, L., Peng, L., Xiaoya, Z., Haoming, L., Ludovic, L., Elizabeth, S., Song, L., Martin, N., & Chengbo, Y. (2020). Effects of a microencapsulated formula of organic acids and essential oils on nutrient absorption, immunity, gut barrier function, and abundance of enterotoxigenic Escherichia coli F4 in weaned piglets challenged with E. coli F4. *Journal of Animal Science*, *98*(9), 1-17. https://doi.org/10.1093/jas/skaa259

Jayaraman, B., & Nyachoti, C. M. (2017). Husbandry practices and gut health outcomes in weaned piglets: a review. *Animal Nutrition*, *3*(3), 205-211. https://doi.org/10.1016/j.aninu.2017.06.002

John, R. P. (2013). Feed- and feed additives-related aspects of gut health and development in weanling pigs. *Journal of Animal Science and Biotechnology*, *4*(2), 83-89. https://doi.org/10.1186/2049-1891-4-1

Kamada, N., Seo, S.-U., Chen, G. Y., & Núñez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology*, *13*(5), 321-335. https://doi.org/10.1038/nri3430

Karimzadeh, S., Rezaei, M., & Teimouri Yansari, A. (2016). Effects of canola bioactive peptides on performance, digestive enzyme activities, nutrient digestibility, intestinal morphology and gut microflora in broiler chickens. *Poultry Science Journal, 4*(1), 27-36.

Kátia Maria, C., Ines, A., Marcos Kipper da, S., Thais Bastos, S., Bruna, S., & Andréa Machado Leal, R. (2021). Estimation of productive losses caused by withdrawal of antibiotic growth promoter from pig diets – Meta-analysis. *Scientia Agricola*, *78*(suppl 1). https://doi.org/10.1590/1678-992x-2020-0266

Keimer, B., Pieper, R., Simon, A., & Zentek, J. (2018). Effect of time and dietary supplementation with processed yeasts (Kluyveromyces fragilis) on immunological parameters in weaned piglets. *Animal Feed Science and Technology*, *245*, 136-146. https://doi.org/10.1016/j.anifeedsci.2018.09.008

Khafipour, E., Munyaka, P. M., Nyachoti, C. M., Krause, D. O., & Rodriguez-Lecompte, J. C. (2014). Effect of crowding stress and Escherichia coli K88+ challenge in nursery pigs supplemented with anti-Escherichia coli K88+ probiotics. *Journal of Animal Science*, *92*(5), 2017-2029. https://doi.org/10.2527/jas.2013-7043

Khajali, F., & Slominski, B. A. (2012). Factors that affect the nutritive value of canola meal for poultry. *Poultry Science*, *91*(10), 2564-2575. https://doi.org/10.3382/ps.2012-02332

Kiarie, E. (2008). Dietary means for enhanced gastrointestinal health and function in weaned pigs : An evaluation of carbohydrase enzymes targeting non-starch polysaccharides. *Thesis (Ph.D.)--University of Manitoba,* May 2008.

Kiarie, E. G. U. o. M. W., Slominski, B. A., & Nyachoti, C. M. (2010). Effect of products derived from hydrolysis of wheat and flaxseed non starch polysaccharides by carbohydrase enzymes on net absorption in enterotoxigenic Escherichia coli (K88) challenged piglet jejunal segments. *Animal Science Journal*, *81*(1), 63-71. https://doi.org/10.1111/j.1740-0929.2009.00716.x

Kim, K., Ehrlich, A., Perng, V., Chase, J. A., Raybould, H., Li, X., Atwill, E. R., Whelan, R., Sokale, A., & Liu, Y. (2019). Algae-derived β-glucan enhanced gut health and immune responses of weaned pigs experimentally infected with a pathogenic E. coli. *Animal Feed Science and Technology*, *248*, 114-125. https://doi.org/10.1016/j.anifeedsci.2018.12.004

Kim, K., He, Y., Jinno, C., Yang, S., Li, X., Bravo, D., Cox, E., & Liu, Y. (2021). 187 Effects of oligosaccharide-based polymer on growth performance, diarrhea, and fecal β-hemolytic Coliforms in weanling pigs experimentally infected with a pathogenic E. coli. *Journal of Animal Science*, *99*(Supplement_1), 90-90. https://doi.org/10.1093/jas/skab054.145

Kluess, J., Schoenhusen, U., Souffrant, W. B., Jones, P. H., & Miller, B. G. (2010). Impact of diet composition on ileal digestibility and small intestinal morphology in early-weaned pigs fitted with a T-cannula. *Animal*, *4*(4), 586-594. https://doi.org/10.1017/S1751731109991455

Kolida, S., Tuohy, K., & Gibson, G. R. (2002). Prebiotic effects of inulin and oligofructose. *British Journal of Nutrition*, *87*(S2), S193-S197. https://doi.org/10.1079/BJN/2002537

Kong, S., Zhang, Y. H., & Zhang, W. (2018). Regulation of intestinal epithelial cells properties and functions by amino acids. *BioMed Research International*, *2018*, 2819154-2819110. https://doi.org/10.1155/2018/2819154

Konstantinov, S. R., Awati, A. A., Williams, B. A., Miller, B. G., Jones, P., Stokes, C. R., Akkermans, A. D. L., Smidt, H., & De Vos, W. M. (2006). Post-natal development of the porcine microbiota composition and activities. *Environmental Microbiology*, *8*(7), 1191-1199. https://doi.org/10.1111/j.1462-2920.2006.01009.x

Koo, B., Bustamante-García, D., Kim, J. W., & Nyachoti, C. M. (2020). Health-promoting effects of Lactobacillus-fermented barley in weaned pigs challenged with Escherichia coli K88. *Animal*, *14*(1), 39-49. https://doi.org/10.1017/S1751731119001939

Koo, B., Choi, J., Yang, C., & Nyachoti, C. M. (2020). Diet complexity and l-threonine supplementation: effects on growth performance, immune response, intestinal barrier function, and microbial metabolites in nursery pigs. *Journal of Animal Science*, *98*(5). https://doi.org/10.1093/jas/skaa125

Koo, B., Hossain, M. M., & Nyachoti, C. M. (2017). Effect of dietary wheat bran inclusion on nutrient and energy digestibility and microbial metabolites in weaned pigs. *Livestock Science*, *203*, 110-113. https://doi.org/10.1016/j.livsci.2017.07.011

Lallès, J.-P., Boudry, G., Favier, C., Le Floc'h, N., Luron, I., Montagne, L., Oswald, I. P., Pié, S., Piel, C., & Sève, B. (2004). Gut function and dysfunction in young pigs: physiology. *Animal Research*, *53*(4), 301-316. https://doi.org/10.1051/animres:2004018

Landers, T. F., Cohen, B., Wittum, T. E., & Larson, E. L. (2012). A review of antibiotic use in food animals: perspective, policy, and potential. *Public Health Reports (1974)*, *127*(1), 4-22. https://doi.org/10.1177/003335491212700103

Lee, J., Kim, J. W., Hall, H. N., & Nyachoti, C. M. (2021). Effect of dietary organic acids supplementation on growth performance, nutrient digestibility, and gut morphology in weaned pigs. *Canadian Journal of Animal Science*. https://doi.org/10.1139/CJAS-2021-0080

Lee, J. W., Patterson, R., & Woyengo, T. A. (2018). Porcine in vitro degradation and fermentation characteristics of canola co-products without or with fiber-degrading enzymes. *Animal Feed Science and Technology*, *241*, 133-140. https://doi.org/10.1016/j.anifeedsci.2018.04.019

Lee, S. I., & Kang, K. S. (2017). Function of capric acid in cyclophosphamide-induced intestinal inflammation, oxidative stress, and barrier function in pigs. *Scientific Reports*, *7*(1), 16530-16512. https://doi.org/10.1038/s41598-017-16561-5

Lewis, M. C., Patel, D. V., Fowler, J., Duncker, S., Zuercher, A. W., Mercenier, A., & Bailey, M. (2013). Dietary supplementation with Bifidobacterium lactis NCC2818 from weaning reduces local immunoglobulin production in lymphoid-associated tissues but increases systemic antibodies in healthy neonates. *British Journal of Nutrition*, *110*(7), 1243-1252. https://doi.org/10.1017/S0007114513000251

Li, X.-Y., Jin, L.-J., Uzonna, J. E., Li, S.-Y., Liu, J.-J., Li, H.-Q., Lu, Y.-N., Zhen, Y.-H., & Xu, Y.-P. (2009). Chitosan–alginate microcapsules for oral delivery of egg yolk immunoglobulin (IgY): In vivo evaluation in a pig model of enteric colibacillosis. *Veterinary Immunology and Immunopathology*, *129*(1), 132-136. https://doi.org/10.1016/j.vetimm.2008.12.016

Li, Y., Song, Z., Kerr, K. A., & Moeser, A. J. (2017). Chronic social stress in pigs impairs intestinal barrier and nutrient transporter function, and alters neuro-immune mediator and receptor expression. *PloS One*, *12*(2), e0171617-e0171617. https://doi.org/10.1371/journal.pone.0171617

Liao, S. F., & Nyachoti, M. (2017). Using probiotics to improve swine gut health and nutrient utilization. *Animal Nutrition*, *3*(4), 331-343. https://doi.org/10.1016/j.aninu.2017.06.007

Liu, P., Piao, X. S., Thacker, P. A., Zeng, Z. K., Li, P. F., Wang, D., & Kim, S. W. (2010). Chito-oligosaccharide reduces diarrhea incidence and attenuates the immune response of weaned pigs challenged with Escherichia coli K88. *Journal of Animal Science*, *88*(12), 3871-3879. https://doi.org/10.2527/jas.2009-2771

Liu, X., Le Bourvellec, C., & Renard, C. M. G. C. (2020). Interactions between cell wall polysaccharides and polyphenols: effect of molecular internal structure. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 3574-3617. https://doi.org/10.1111/1541-4337.12632

Liu, Y., Song, M., Che, T. M., Almeida, J. A. S., Lee, J. J., Bravo, D., Maddox, C. W., & Pettigrew, J. E. (2013). Dietary plant extracts alleviate diarrhea and alter immune responses of weaned pigs experimentally infected with a pathogenic Escherichia coli. *Journal of Animal Science*, *91*(11), 5294-5306. https://doi.org/10.2527/jas.2012-6194

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using Real-Time Quantitative PCR and the 2−ΔΔCT method. *Methods, 25*(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Long, S., Hu, J., Mahfuz, S., Ma, H., & Piao, X. (2021). Effects of dietary supplementation of compound enzymes on performance, nutrient digestibility, serum antioxidant status, immunoglobulins, intestinal morphology and microbiota community in weaned pigs. *Archives of Animal Nutrition*, *75*(1), 31-47. https://doi.org/10.1080/1745039X.2020.1852008

Luise, D., Bertocchi, M., Motta, V., Salvarani, C., Bosi, P., Luppi, A., Fanelli F., Mazzoni M., Archetti I., Maiorano G., Nielsen B., & Trevisi, P. (2019). Bacillus sp. probiotic supplementation diminish the Escherichia coli F4ac infection in susceptible weaned pigs by influencing the intestinal immune response, intestinal microbiota and blood metabolomics. Journal of Animal Science and Biotechnology, 10(1), 1-16. https://doi.org/10.1186/s40104-020-00482-x

Luise, D., Lauridsen, C., Bosi, P., & Trevisi, P. (2019). Methodology and application of Escherichia coli F4 and F18 encoding infection models in post-weaning pigs. *Journal of Animal Science and Biotechnology*, *10*(1), 53-53. https://doi.org/10.1186/s40104-019-0352-7

Luying, P., Li, Z.-R., Green, R. S., Holzman, I. R., & Jing, L. I. N. (2009). Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-Activated protein kinase in Caco-2 cell monolayers. *The Journal of Nutrition*, *139*(9), 1619-1625. https://doi.org/10.3945/jn.109.104638

Macfarlane, G. T., Steed, H., & Macfarlane, S. (2008). Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *Journal of Applied Microbiology*, *104*(2), 305-344. https://doi.org/10.1111/j.1365-2672.2007.03520.x

Mach, N., Berri, M., Estellé, J., Levenez, F., Lemonnier, G., Denis, C., Leplat, J.-J., Chevaleyre, C., Billon, Y., Doré, J., Rogel-Gaillard, C., & Lepage, P. (2015). Early-life establishment of the swine gut microbiome and impact on host phenotypes. *Environmental Microbiology Reports*, *7*(3), 554-569. https://doi.org/10.1111/1758-2229.12285

Marín-Manzano, M. C., Abecia, L., Hernández-Hernández, O., Sanz, M. L., Montilla, A., Olano, A., Rubio, L. A., Moreno, F. J., & Clemente, A. (2013). Galacto-oligosaccharides derived from lactulose exert a selective stimulation on the growth of Bifidobacterium animalis in the large intestine of growing rats. *Journal of Agricultural and Food Chemistry*, *61*(31), 7560-7567. https://doi.org/10.1021/jf402218z

Marquardt, R. R., Jin, L. Z., Kim, J.-W., Fang, L., Frohlich, A. A., & Baidoo, S. K. (1999). Passive protective effect of egg-yolk antibodies against enterotoxigenic Escherichia coli K88+ infection in neonatal and early-weaned piglets. *FEMS Immunology and Medical Microbiology*, *23*(4), 283-288. https://doi.org/10.1111/j.1574-695X.1999.tb01249.x

Mathlouthi, N., Mohamed, M. A., & Larbier, M. (2003). Effect of enzyme preparation containing xylanase and ß-glucanase on performance of laying hens fed wheat/barley- or maize/soybean meal-based diets. *British Poultry Science*, *44*(1), 60-66. https://doi.org/10.1080/0007166031000085374

Mejicanos, G. A. (2019). Optimizing the use of canola meal in swine diets for lifetime performance. *Thesis (Ph.D.)--University of Manitoba,*2019

Mengíbar, M., Mateos-Aparicio, I., Miralles, B., & Heras, Á. (2013). Influence of the physico-chemical characteristics of chito-oligosaccharides (COS) on antioxidant activity. *Carbohydrate Polymers*, *97*(2), 776-782. https://doi.org/10.1016/j.carbpol.2013.05.035

Messori, S., Trevisi, P., Simongiovanni, A., Priori, D., & Bosi, P. (2013). Effect of susceptibility to enterotoxigenic Escherichia coli F4 and of dietary tryptophan on gut microbiota diversity observed in healthy young pigs. *Veterinary Microbiology*, *162*(1), 173-179. https://doi.org/10.1016/j.vetmic.2012.09.001

Mikkelsen, L. L., Jakobsen, M., & Jensen, B. B. (2003). Effects of dietary oligosaccharides on microbial diversity and fructo-oligosaccharide degrading bacteria in faeces of piglets post-weaning. *Animal Feed Science and Technology*, *109*(1), 133-150. https://doi.org/10.1016/S0377-8401(03)00172-X

Molist, F., van Oostrum, M., Pérez, J. F., Mateos, G. G., Nyachoti, C. M., & van der Aar, P. J. (2014). Relevance of functional properties of dietary fibre in diets for weanling pigs. *Animal Feed Science and Technology*, *189*, 1-10. https://doi.org/10.1016/j.anifeedsci.2013.12.013

Mrabti, H. N., El Abbes Faouzi, M., Mayuk, F. M., Makrane, H., Limas-Nzouzi, N., Dibong, S. D., Cherrah, Y., Elombo, F. K., Gressier, B., Desjeux, J.-F., & Eto, B. (2019). Arbutus unedo L., (Ericaceae) inhibits intestinal glucose absorption and improves glucose tolerance in rodents. *Journal of Ethnopharmacology*, *235*, 385-391. https://doi.org/10.1016/j.jep.2019.02.013

Naqid, I. A., Owen, J. P., Maddison, B. C., Gardner, D. S., Foster, N., Tchórzewska, M. A., La Ragione, R. M., & Gough, K. C. (2015). Prebiotic and probiotic agents enhance antibody-based immune responses to Salmonella Typhimurium infection in pigs. *Animal Feed Science and Technology*, *201*, 57-65. https://doi.org/10.1016/j.anifeedsci.2014.12.005

Navarro, D. M. D. L., Abelilla, J. J., & Stein, H. H. (2019). Structures and characteristics of carbohydrates in diets fed to pigs: a review. *Journal of Animal Science and Biotechnology*, *10*(1), 39-39. https://doi.org/10.1186/s40104-019-0345-6

Ndou, S. P. (2018). Production, absorption, and metabolic fate of fatty acids in pigs fed high-fiber diets. *Thesis (Ph.D.)--University of Manitoba,* Dec 2008

Nielsen, T. S., Lærke, H. N., Theil, P. K., Sørensen, J. F., Saarinen, M., Forssten, S., & Bach Knudsen, K. E. (2014). Diets high in resistant starch and arabinoxylan modulate digestion processes and SCFA pool size in the large intestine and faecal microbial composition in pigs. *British Journal of Nutrition*, *112*(11), 1837-1849. https://doi.org/10.1017/S000711451400302X

Niewold, T. A. (2007). The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. *Poultry Science*, *86*(4), 605-609. https://doi.org/10.1093/ps/86.4.605

Novozamsky, V. (1974). The shape of weightbearing plantar arches in various positions (author's transl). *Zeitschrift für Orthopädie und ihre Grenzgebiete*, *112*(5), 1137-1142.

NRC. (2012). *Nutrient requirements of swine* ([11th rev.] ed.). National Academies Press.

Oh, H. K., Choi, H. B., Ju, W. S., Chung, C. S., & Kim, Y. Y. (2010). Effects of space allocation on growth performance and immune system in weaning pigs. *Livestock Science*, *132*(1), 113-118. https://doi.org/10.1016/j.livsci.2010.05.009

Olivier, M., Berthon, P., & Salmon, H. (1994). Immunohistochemical localization of the humoral and cellular components of the immune response in swine gut. *Veterinary Research*, *25*(1).

Omonijo, F., Kim, S., Guo, T., Wang, Q., Gong, J., Lahaye, L., Bodin, J.-C., Nyachoti, M., Liu, S., & Yang, C. (2018). Development of novel microparticles for effective delivery of thymol and lauric acid to pig intestinal tract. *Journal of Agriculture and Food Chemistry, 66*(37), 9608-9615. https://doi.org/10.1021/acs.jafc.8b02808

Park, J.-H., Lee, S.-I., & Kim, I.-H. (2018). Effect of dietary β-glucan supplementation on growth performance, nutrient digestibility, and characteristics of feces in weaned pigs. *Journal of Applied Animal Research*, *46*(1), 1193-1197. https://doi.org/10.1080/09712119.2018.1481855

Park, S., Lee, J. W., Jerez Bogota, K., Francis, D., González-Vega, J. C., Htoo, J. K., & Woyengo, T. A. (2020). Growth performance and gut health of Escherichia coli-challenged weaned pigs fed diets supplemented with a Bacillus subtilis direct-fed microbial. *Translational Animal Science*, *4*(3), txaa172-txaa172. https://doi.org/10.1093/tas/txaa172

Paternostre, L., De Boever, J., & Millet, S. (2021). Interaction between fat and fiber level on nutrient digestibility of pig feed. *Animal Feed Science and Technology*, *282*, 115126. https://doi.org/10.1016/j.anifeedsci.2021.115126

Petri, D., Hill, J. E., & Van Kessel, A. G. (2010). Microbial succession in the gastrointestinal tract (GIT) of the preweaned pig. *Livestock Science*, *133*(1), 107-109. https://doi.org/10.1016/j.livsci.2010.06.037

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*(9), 45e-45. https://doi.org/10.1093/nar/29.9.e45

Pie, S., Lalles, J. P., Blazy, F., Laffitte, J., Seve, B., & Oswald, I. P. (2004). Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. *The Journal of Nutrition*, *134*(3), 641-647. https://doi.org/10.1093/jn/134.3.641

Pluske, J. R., Pethick, D. W., Hopwood, D. E., & Hampson, D. J. (2002). Nutritional influences on some major enteric bacterial diseases of pigs. *Nutrition Research Reviews*, *15*(2), 333-371.

Pluske, J. R., Turpin, D. L., & Kim, J.-C. (2018). Gastrointestinal tract (gut) health in the young pig. *Animal Nutrition, 4*(2), 187-196. https://doi.org/10.1016/j.aninu.2017.12.004

Pollet, A., Van Craeyveld, V., Van de Wiele, T., Verstraete, W., Delcour, J. A., & Courtin, C. M. (2012). In vitro fermentation of arabinoxylan oligosaccharides and low molecular mass arabinoxylans with different structural properties from wheat (Triticum aestivum L.) Bran and Psyllium (Plantago ovata Forsk) seed husk. *Journal of Agricultural and Food Chemistry*, *60*(4), 946-954. https://doi.org/10.1021/jf203820j

Poudel, P., Levesque, C. L., Samuel, R., & St-Pierre, B. (2020). Dietary inclusion of Peptiva, a peptide-based feed additive, can accelerate the maturation of the fecal bacterial

microbiome in weaned pigs. *BMC Veterinary Research*, *16*(1), 60-60. https://doi.org/10.1186/s12917-020-02282-x

Pourabedin, M., & Zhao, X. (2015). Prebiotics and gut microbiota in chickens. *FEMS Microbiology Letters*, *362*(15), fnv122-fnv122. https://doi.org/10.1093/femsle/fnv122

Qaisrani, S. N., Moquet, P. C. A., Krimpen, v. M. M., Kwakkel, R. P., Verstegen, M. W. A., & Heniks, W. H. (2014). Protein source and dietary structure influence growth performance, gut morphology, and hindgut fermentation characteristics in broilers. *Poultry Science*, *93*(12), 3053-3064. https://doi.org/10.3382/ps.2014-04091

Radfar, M., Rogiewicz, A., & Slominski, B. A. (2017). Chemical composition and nutritive value of canola-quality Brassica juncea meal for poultry and the effect of enzyme supplementation. *Animal Feed Science and Technology*, *225*, 97-108. https://doi.org/10.1016/j.anifeedsci.2017.01.007

Read, L. T., Hahn, R. W., Thompson, C. C., Bauer, D. L., Norton, E. B., & Clements, J. D. (2014). Simultaneous exposure to Escherichia coli heat-labile and heat-stable enterotoxins increases fluid secretion and alters cyclic nucleotide and cytokine production by intestinal epithelial cells. *Infection and Immunity*, *82*(12), 5308-5316. https://doi.org/10.1128/IAI.02496-14

Ryan, J. P. (1980). Determination of volatile fatty acids and some related compounds in ovine rumen fluid, urine, and blood plasma, by gas-liquid chromatography. *Analytical Biochemistry*, *108*(2), 374-384. https://doi.org/10.1016/0003-2697(80)90602-8

Sánchez, B., Delgado, S., Blanco-Míguez, A., Lourenço, A., Gueimonde, M., & Margolles, A. (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Molecular Nutrition & Food Research*, *61*(1), 1600240-n/a. https://doi.org/10.1002/mnfr.201600240

Scharek-Tedin, L., Pieper, R., Vahjen, W., Tedin, K., Neumann, K., & Zentek, J. (2013). Bacillus cereus var. Toyoi modulates the immune reaction and reduces the occurrence of diarrhea in piglets challenged with Salmonella Typhimurium DT104. *Journal of Animal Science*, *91*(12), 5696-5704. https://doi.org/10.2527/jas.2013-6382

Scott, R. W. (1979). Colorimetric determination of hexuronic acids in plant materials. *Analytical Chemistry, 51*(7), 936-941. https://doi.org/10.1021/ac50043a036

Silva, C. A., Silva, Dias, C. P., Callegari, M. A., Bridi, A. M., Santos, R. K. S., Luiggi, F. G., Santos, V. L., & Silva, J. B. (2020). Prebiotics and butyric acid can replace colistin as a growth promoter for nursery piglets. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia,* 72(4), 1449–1457. https://doi.org/10.1590/1678-4162-11596

Sinha, R., Sahoo, N. R., Shrivastava, K., Kumar, P., Qureshi, S., De, U. K., Kumar, A., Kumar, G. V. P. P. S. R., & Bhushan, B. (2019). Resistance to ETEC F4/F18–mediated piglet diarrhoea: opening the gene black box. *Tropical Animal Health and Production*, *51*(6), 1307-1320. https://doi.org/10.1007/s11250-019-01934-x

Slavin, J. (2013). Fiber and prebiotics: mechanisms and health benefits. *Nutrients*, *5*(4), 1417-1435. https://doi.org/10.3390/nu5041417

Slominski, B. A., Guenter, W., & Campbell, L. D. (1993). New approach to water-soluble carbohydrate determination as a tool for evaluation of plant cell wall-degrading enzymes. *Journal of Agricultural and Food Chemistry*, *41*(12), 2304-2308. https://doi.org/10.1021/jf00036a016

Slominski, B. A., Meng, X., Campbell, L. D., Guenter, W., & Jones, O. (2006). The use of enzyme technology for improved energy utilization from full-fat oilseeds. Part II: flaxseed. *Poultry Science*, *85*(6), 1031-1037. https://doi.org/10.1093/ps/85.6.1031

Spreeuwenberg, M. A., Verdonk, J. M., Gaskins, H. R., & Verstegen, M. W. A. (2001). Small intestine epithelial barrier function is compromised in pigs with low feed intake at weaning. *The Journal of Nutrition*, *131*(5), 1520-1520. https://doi.org/10.1093/jn/131.5.1520

Stokes, C. R., Bailey, M., Sowa, E., RothkÖTter, H.-J., Miller, B. G., Haverson, K., Harris, C., Jones, P., Inman, C., Pie, S., Oswald, I. P., Williams, B. A., & Akkermans, A. D. L. (2004). Postnatal development of intestinal immune system in piglets: implications for the process of weaning. *Animal Research 53*(4),325-4. https://doi.org/10.1051/animres:2004020

Su, G., Zhou, X., Wang, Y., Chen, D., Chen, G., Li, Y., & He, J. (2020). Dietary supplementation of plant essential oil improves growth performance, intestinal morphology and health in weaned pigs. *Journal of Animal Physiology and Animal Nutrition*, *104*(2), 579-589. https://doi.org/10.1111/jpn.13271

Suiryanrayna, M. V. A. N., & Ramana, J. V. (2016). A review of the effects of dietary organic acids fed to swine. *Journal of Animal Science and Biotechnology*, *7*(1), 67-77. https://doi.org/10.1186/s40104-015-0042-z

Sun, Y., & Kim, S. W. (2017). Intestinal challenge with enterotoxigenic Escherichia coli in pigs, and nutritional intervention to prevent postweaning diarrhea. *Animal Nutrition*, *3*(4), 322-330. https://doi.org/10.1016/j.aninu.2017.10.001

Tarr, P. I., Gordon, C. A., & Chandler, W. L. (2005). Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. *The Lancet, 365*(9464), 1073-1086. https://doi.org/10.1016/S0140-6736(05)71144-2

Thomas, D. M., Walker, P. D., Benjamins, J. A., Geddes, T. J., & Kuhn, D. M. (2004). Methamphetamine neurotoxicity in dopamine nerve endings of the striatum is associated with microglial activation. *The Journal of Pharmacology and Experimental Therapeutics*, *311*(1), 1-7. https://doi.org/10.1124/jpet.104.070961

Tiwari, U. P., Fleming, S. A., Abdul Rasheed, M. S., Jha, R., & Dilger, R. N. (2020). The role of oligosaccharides and polysaccharides of xylan and mannan in gut health of monogastric animals. *Journal of Nutritional Science*, *9*, e21-e21. https://doi.org/10.1017/jns.2020.14

Tohno, M., Shimosato, T., Kitazawa, H., Katoh, S., Iliev, I. D., Kimura, T., Kawai, Y., Watanabe, K., Aso, H., Yamaguchi, T., & Saito, T. (2005). Toll-like receptor 2 is expressed on the intestinal M cells in swine. *Biochemical and Biophysical Research Communications*, *330*(2), 547-554. https://doi.org/10.1016/j.bbrc.2005.03.017

Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A., & Warman, M. L. (2000). Preparation of PCR-Quality mouse genomic DNA with hot sodium hydroxide and tris (HotSHOT). *BioTechniques*, *29*(1), 52-54. https://doi.org/10.2144/00291bm09

Tsukahara, T., Kimura, Y., Inoue, R., & Iwata, T. (2020). Preliminary investigation of the use of dietary supplementation with probiotic Bacillus subtilis strain QST713 shows that it attenuates antimicrobial-induced dysbiosis in weaned piglets. *Animal Science Journal*, *91*(1), e13475-n/a. https://doi.org/10.1111/asj.13475

Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G., Gilbert, M., Bonhoeffer, S., & Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science*, *365*(6459), 1266. https://doi.org/10.1126/science.aaw1944

Vondruskova, H. V. U. V. L. B., Slamova, R. V. U. V. L. B., Trckova, M. V. U. V. L. B., Zraly, Z. V. U. V. L. B., & Pavlik, I. V. U. V. L. B. (2010). Alternatives to antibiotic growth

promoters in prevention of diarrhoea in weaned piglets: a review. *Veterinární Medicína*, *55*(5), 199-224. https://doi.org/10.17221/2998-VETMED

Walter, J. (2008). Ecological role of Lactobacilli in the gastrointestinal tract : implications for fundamental and biomedical research. *Applied and Environmental Microbiology*, *74*(16), 4985-4996. https://doi.org/10.1128/AEM.00753-08

Wang, Y., Kuang, Y., Zhang, Y., Song, Y., Zhang, X., Lin, Y., Che, L., Xu, S., Wu, D., Xue, B., & Fang, Z. (2016). Rearing conditions affected responses of weaned pigs to organic acids showing a positive effect on digestibility, microflora and immunity. *Animal Science Journal*, *87*(10), 1267-1280. https://doi.org/10.1111/asj.12544

Wohlgemuth, S., Loh, G., & Blaut, M. (2009). Recent developments and perspectives in the investigation of probiotic effects. *International Journal of Medical Microbiology*, *300*(1), 3-10. https://doi.org/10.1016/j.ijmm.2009.08.003

Woolhouse, M., Ward, M., van Bunnik, B., & Farrar, J. (2015). Antimicrobial resistance in humans, livestock and the wider environment. *Philosophical transactions of The Royal Society B: Biological Sciences*, *370*(1670), 20140083-20140083. https://doi.org/10.1098/rstb.2014.0083

Woyengo, T. A., Rodriguez-Lecompte, J. C., Adeola, O., & Nyachoti, C. M. (2011). Histomorphology and small intestinal sodium-dependent glucose transporter 1 gene expression in piglets fed phytic acid and phytase-supplemented diets. *Journal of Animal Science*, *89*(8), 2485-2490. https://doi.org/10.2527/jas.2010-3204

Wu, C., Xu, Q., Wang, R., Qin, L., Peng, X., Hu, L., Liu, Y., Fang, Z., Lin, Y., Xu, S., Feng, B., Li, J., Wu, D., & Che, L. (2018). Effects of dietary β-glucan supplementation on growth performance and immunological and metabolic parameters of weaned pigs administered with Escherichia coli lipopolysaccharide. *Food & function*, *9*(6), 3338-3343. https://doi.org/10.1039/c7fo01980g

Wu, X., Chen, D., Yu, B., Luo, Y., Zheng, P., Mao, X., Yu, J., & He, J. (2018). Effect of different dietary non-starch fiber fractions on growth performance, nutrient digestibility, and intestinal development in weaned pigs. *Nutrition*, *51-52*, 20-28. https://doi.org/10.1016/j.nut.2018.01.011

Xiao, D., Wang, Y., Liu, G., He, J., Qiu, W., Hu, X., Feng, Z., Ran, M., Nyachoti, C. M., Kim, S. W., Tang, Z., & Yin, Y. (2014). Effects of chitosan on intestinal inflammation in weaned

pigs challenged by enterotoxigenic Escherichia coli. *PloS One*, *9*(8), e104192. https://doi.org/10.1371/journal.pone.0104192

Xiao, H., Shao, F., Wu, M., Ren, W., Xiong, X., Tan, B., & Yin, Y. (2016). The application of antimicrobial peptides as growth and health promoters for swine. *Journal of Animal Science and Biotechnology*, *7*(1), 20-25. https://doi.org/10.1186/s40104-015-0018-z

Yadav, S., Teng, P. Y., Choi, J., Singh, A. K., Vaddu, S., Thippareddi, H., & Kim, W. K. (2021). Influence of rapeseed, canola meal and glucosinolate metabolite (AITC) as potential antimicrobials: Effects on growth performance, and gut health in Salmonella Typhimurium challenged broiler chickens. *Poultry Science*, 101551. https://doi.org/10.1016/j.psj.2021.101551

Yang, P., & Zhao, J. (2021). Fiber digestibility in growing pigs fed common fiber-rich ingredients: a systematic review. *Annals of Animal Science*. https://doi.org/10.2478/aoas-2021-0050

Yu, E., Chen, D., Yu, B., Luo, Y., Zheng, P., Yin, H., Mao, X., Huang, Z., Yu, J., Luo, J., HuiYan, & He, J. (2021). Amelioration of enterotoxigenic Escherichia coli-induced disruption of intestinal epithelium by manno-oligosaccharide in weaned pigs. *Journal of Functional Foods*, *82*, 104492. https://doi.org/10.1016/j.jff.2021.104492

Zanello, G., Berri, M., Dupont, J., Sizaret, P.-Y., D'Inca, R., Salmon, H., & Meurens, F. (2011). Saccharomyces cerevisiae modulates immune gene expressions and inhibits ETEC-mediated ERK1/2 and p38 signaling pathways in intestinal epithelial cells. *PloS One*, *6*(4), e18573-e18573. https://doi.org/10.1371/journal.pone.0018573

Zeyner, A., & Boldt, E. (2006). Effects of a probiotic Enterococcus faecium strain supplemented from birth to weaning on diarrhoea patterns and performance of piglets. *Journal of Animal Physiology and Animal Nutrition*, *90*(1-2), 25-31. https://doi.org/10.1111/j.1439-0396.2005.00615.x

Zhang, W., Zhu, Y.-H., Yang, J.-C., Yang, G.-Y., Zhou, D., & Wang, J.-F. (2015). A Selected Lactobacillus rhamnosus Strain Promotes EGFR-Independent Akt Activation in an Enterotoxigenic Escherichia coli K88-Infected IPEC-J2 Cell Model. *PloS One*, *10*(4), e0125717-e0125717. https://doi.org/10.1371/journal.pone.0125717

Zhao, P. Y., & Kim, I. H. (2015). Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in

weanling pigs. *Animal Feed Science and Technology*, *200*, 86-92. https://doi.org/10.1016/j.anifeedsci.2014.12.010

Zhu, J., Yin, X., Yu, H., Zhao, L., Sabour, P., & Gong, J. (2011). Involvement of quorum sensing and heat-stable enterotoxin a in cell damage caused by a porcine enterotoxigenic Escherichia coli strain. *Infection and Immunity*, *79*(4), 1688-1695. https://doi.org/10.1128/IAI.01281-10