REGULATION OF OXIDATIVE STRESS AND INFLAMMATION IN GUT HEALTH

By

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ABSTRACT

The gastrointestinal tract plays an essential role in maintaining overall health. It is vulnerable to oxidative stress that disrupts intestinal integrity, promotes inflammation, and impairs nutrient absorption. Oxidative stress is an imbalance between reactive oxygen species production and antioxidant defense. Glutathione (GSH) is a potent endogenous antioxidant that plays a crucial role in maintaining redox homeostasis and protecting intestinal cells from oxidative damage. The mechanisms by which oxidative stress affects gut health remain poorly understood. This research aimed to examine the impact of oxidative stress on gut health and the underlying mechanisms; and to explore whether dietary fiber and probiotics could alleviate oxidative stress and improve gut health. **Study I** evaluated the effects of high- and low-fiber diets on intestinal oxidative stress in growing-finishing pigs. Compared to the low-fiber diet, the high-fiber diet significantly reduced intestinal oxidative stress biomarkers and improved growth performance. **Study II** examined the effect of *E. coli* infection on intestinal oxidative stress and the mechanisms involved in post-weaning piglets. *E. coli* infection is a major cause of post-weaning diarrhea in piglets. *E. coli* infection suppressed nuclear factor erythroid 2-related factor 2 (Nrf2) signaling, reduced glutathione-synthesizing enzyme expression, and depleted serum and intestinal GSH level, decreased tight junction protein expression, increased intestinal permeability and elevated proinflammatory cytokines expression. Antibiotic treatment alleviated these effects. Supplementation with GSH restored Nrf2 expression, reduced proinflammatory cytokine expression, and mitigated epithelial oxidative damage. **Study III** investigated the effects of probiotic *Bacillus licheniformis* HG76 on *E. coli*-induced oxidative stress in post-weaning piglets. The probiotic restored serum and intestinal GSH

levels and nuclear Nrf2 expression, upregulated glutathione-synthesizing enzymes expression, and reduced lipid peroxidation. It also enhanced tight junction protein expression and lowered proinflammatory cytokine expression. In conclusion, these studies demonstrate that oxidative stress contributes to intestinal dysfunction by suppressing Nrf2 activation and GSH biosynthesis, leading to impaired gut health and growth performance. Dietary fiber and probiotics can enhance antioxidant capacity and maintain intestinal redox balance, thereby serving as effective strategies to mitigate oxidative damage and improve gut health in swine production.

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DEDICATION

This thesis is dedicated to my beloved wife, Yawei Zhao, for her unwavering love, encouragement, and support throughout this journey. Her patience and belief in me have been my greatest source of strength.

I also dedicate this work to my family, whose unconditional love and sacrifices have always inspired me to persevere. Their guidance and faith in my abilities have been a constant source of motivation.

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FOREWORD

This thesis, authored by Shunshun Jin, follows the sandwich thesis format and comprises three multi-authored manuscripts. All manuscripts have been reviewed, revised, and approved by the respective co-authors.

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LIST OF ABBREVIATIONS

ABX	Antibiotic
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
BW	Boday weight
CON	Control
DAO	Diamine oxidase
DE	Digestible energy
ETEC	Enterotoxigenic *Escherichia coli*
FCR	Feed conversion ratio
GCL	Glutamate-cysteine ligase
Gclc	Glutamate-cysteine ligase catalytic subunit
Cclm	Glutamate-cysteine ligase modifier subunit
GLUT2	Glucose transporter 2
GLUT5	Glucose transporter 5
GS	Glutathione synthetase
GSH	Reduced glutathione
GSH-Px	Glutathione peroxidase
GSSG	Oxidized glutathione
HF	High-fiber
HO-1	Heme oxygenase-1
IgA	Immunoglobulin A

LF	Low-fiber
MDA	Malondialdehyde
ME	Metabolizable energy
NDF	Neutral detergent fiber
NE	Net energy
NF-κB	Nuclear factor kappa B
Nrf2	Nuclear factor erythroid 2-related factor 2
NSP	Non-starch polysaccharides
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PRO	Probiotic
ROS	Reactive oxygen species
SCFAs	Short-chain fatty acids
SD	Standard deviation
SEM	Standard error of the mean
SGLT1	Sodium-glucose linked transporter 1
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
ZO-1	Zonula occludens-1

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Manuscripts

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Figures

Figure 1.1: Composition and structure of small intestine and large intestine. Dmytriv, T. R., Storey, K. B., & Lushchak, V. I. (2024). Intestinal barrier permeability: the influence of gut microbiota, nutrition, and exercise. Frontiers in Physiology, 15, 1380713. Doi: 10.3389/fphys.2024.1380713. Reproduced with permission from MDPI which permits use of content under Creative Commons Attribution License.

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Figure 1.6: The positive impacts of dietary fiber on swine gut health. Megur, A., Daliri, E. B. M., Baltriukienė, D., & Burokas, A. (2022). Prebiotics as a tool for the prevention and treatment of obesity and diabetes: classification and ability to modulate the gut microbiota. International journal of molecular sciences, 23(11), 6097. Doi: 10.3390/ijms23116097. Reproduced with permission from MDPI which permits use of content under Creative Commons Attribution License.

Figure 1.7: The impacts of enterotoxigenic *Escherichia coli* (ETEC) on swine gut health. Kim, K., Song, M., Liu, Y., & Ji, P. (2022b). Enterotoxigenic *Escherichia coli* infection of weaned pigs: Intestinal challenges and nutritional intervention to enhance disease resistance. Frontiers in Immunology, 13, 885253. Doi: 10.3389/fimmu.2022.885253. Reproduced with permission from Frontiers which permits use of content under Creative Commons Attribution License.

CHAPTER ONE: LITERATURE REVIEW

1.1 General introduction

The maintenance of intestinal health is the primary factor of effective nutrient uptake, immunity, and overall health in both animals and humans (Jacobi and Odle, 2012; De Santis et al., 2015). The intestine plays the role not only as a digestion and absorption site but also as a barrier which keeps pathogenic microorganisms, toxins, and antigens from the systemic circulation (Martel et al., 2022; Tang et al., 2022). A balanced gut environment is determined by tight junctions of the epithelial cell membranes, a proper microbial population, and a normal immune response (Peterson and Artis, 2014; Panwar et al., 2021). Disturbances in the gut environment lead to impaired growth, reduced feed efficiency, and increased susceptibility to diseases (Upadhaya and Kim, 2021). Among the factors that disrupt intestinal stability, oxidative stress is of increasing interest, which is related to its enormous impact on intestinal function and health status (Vona et al., 2021). It is believed that oxidative stress happens when the production of reactive oxidative species (ROS) is higher than the detoxifying capacity of endogenous antioxidants within the body, resulting in lipid peroxidation, protein oxidation, and DNA damage (Mirończuk-Chodakowska et al., 2018). In the intestine, the higher levels of ROS damage tight junction proteins, such as occludin and zonula occludens-1 (ZO-1), disrupting the structure of the intestinal epithelium and allowing the dissemination of pathogens and endotoxins into the bloodstream (Rogers et al., 2023). The transport of these pro-inflammatory components across the intestinal barrier can worsen oxidative stress and create a feedback loop of inflammatory reactions (Lin et al., 2022). Some studies have shown that oxidative stress can reduce nutrient uptake, alter immune response, and impair growth performance in livestock (Yin et al., 2015; Chen et al., 2021a). Oxidative stress also leads to chronic

gastrointestinal disorders, malabsorption, and inflammatory bowel diseases in humans (Bourgonje et al., 2020; Oroian et al., 2021). Therefore, the ability to manage oxidative stress is crucial for preventing intestinal dysfunction, achieving better animal productivity, and human health.

Due to the role of oxidative stress in the intestine, a variety of strategies have been developed to restore antioxidant levels and manage gut barrier function (Mo et al., 2022; Yan et al., 2024). One such nutritional strategy involves adjusting dietary fiber. Previous studies established that dietary fiber could alter gut fermentation patterns, increasing short-chain fatty acids (SCFAs) and supporting beneficial microbial populations (Nogal et al., 2021; Zheng et al., 2024). SCFAs such as butyrate have been shown to improve tight junction strength and stimulate antioxidant defense systems, potentially mitigating ROS-induced damage (Russo et al., 2012; Wang et al., 2012). Despite these findings, the precise molecular mechanisms through which fiber improve redox balance remain unclear. Glutathione (GSH) is a crucial endogenous antioxidant that directly neutralizes ROS and preserves cellular redox balance (Kidd, 1997). Limited evidence exists on how fiber affects the regulatory pathways that govern glutathione biosynthesis. This lack of mechanistic insight hinders the development of precise fiber-based interventions that could consistently enhance antioxidant defenses, reduce oxidative damage, and ultimately improve intestinal health and productivity.

In addition to dietary factors, intestinal oxidative stress can be caused by pathogenic infections, with enterotoxigenic *Escherichia coli* (ETEC) being a classic example in pigs.

ETEC infection commonly causes diarrhea and reduced performance in young animals, particularly weaned piglets, and poses a considerable burden in both livestock production and human health (Lee et al., 2017; Luise et al., 2020; von Mentzer and Svennerholm, 2024). While the initial effects of ETEC are often related to fluid secretion and nutrient malabsorption, growing evidence shows that ETEC infection leads to redox imbalance (Tang et al., 2019). Such redox imbalances compromise tight junction proteins and drive systemic inflammation (Duan et al., 2022). The maintenance of GSH homeostasis is influenced by regulatory factors that respond to changes in redox status. Among these regulators, transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) plays a key role. Few studies are focused on the role of Nrf2 in regulating antioxidant responses and the enzymatic steps involved in GSH depletion under ETEC challenge. Furthermore, it remains unclear whether mitigating oxidative stress can also help restore tight junction integrity and improve overall gut health. Although antibiotics often reduce pathogen load during ETEC infection, overuse raises concerns about resistance and long-term sustainability. These limitations highlight the need for alternative methods that restore antioxidant capacity, improve barrier integrity, and minimize inflammatory damage.

Probiotics offer a promising non-antibiotic approach to reinforce intestinal antioxidant defenses. They have gained attention for their capacity to improve gut health by modulating microbiota composition, enhancing immune responses, and potentially strengthening antioxidant defenses (Sáez-Lara et al., 2016; Bron et al., 2017; Plaza-Diaz et al., 2019). Some probiotics have been shown to reduce intestinal malondialdehyde (MDA), increase GSH content, and improve barrier integrity, aligning well with the goal of mitigating

oxidative stress (Petrova et al., 2021; Palkovicsné Pézsa et al., 2022). *Bacillus licheniformis*, a robust spore-forming bacterium, has emerged as a promising candidate in this regard. Previous studies have shown that *Bacillus licheniformis* supplementation increased antioxidant enzyme activities in the intestine and reduced indicators of oxidative damage (Yu et al., 2022b; Qin et al., 2024a). However, despite these encouraging findings under normal conditions, the effectiveness and mechanisms of *Bacillus licheniformis* in restoring antioxidant capacity during ETEC challenge remain insufficiently understood.

To address these gaps, three complementary studies were conducted. Study 1 focused on dietary fiber and its effects on intestinal oxidative stress and redox balance. Study 2 revolved around the ETEC infection model to characterize oxidative stress-related mechanisms in the gut. Study 3 specifically examined *Bacillus licheniformis* supplementation in the context of ETEC-induced oxidative stress. Building on earlier evidence that certain probiotics improve antioxidant status, this study aimed to confirm whether *Bacillus licheniformis* can restore GSH synthesis, enhance Nrf2-dependent antioxidant enzyme expression, and reduce ROS accumulation and inflammation under ETEC infection conditions.

1.2 Intestinal structure and function

1.2.1 Intestinal structure

The intestine is a complex organ that not only has the ability to digest and absorb nutrients but is also the largest immune organ. The intestine is composed of multiple layers of special structures, which are mainly divided into mucosa (epithelium, lamina propria, muscularis

mucosae), submucosa and muscularis externa. Together, they form a hollow tube around the central lumen. The intestine is mainly divided into two parts: the large intestine and the small intestine. Their composition and structure are shown in Figure 1.1.

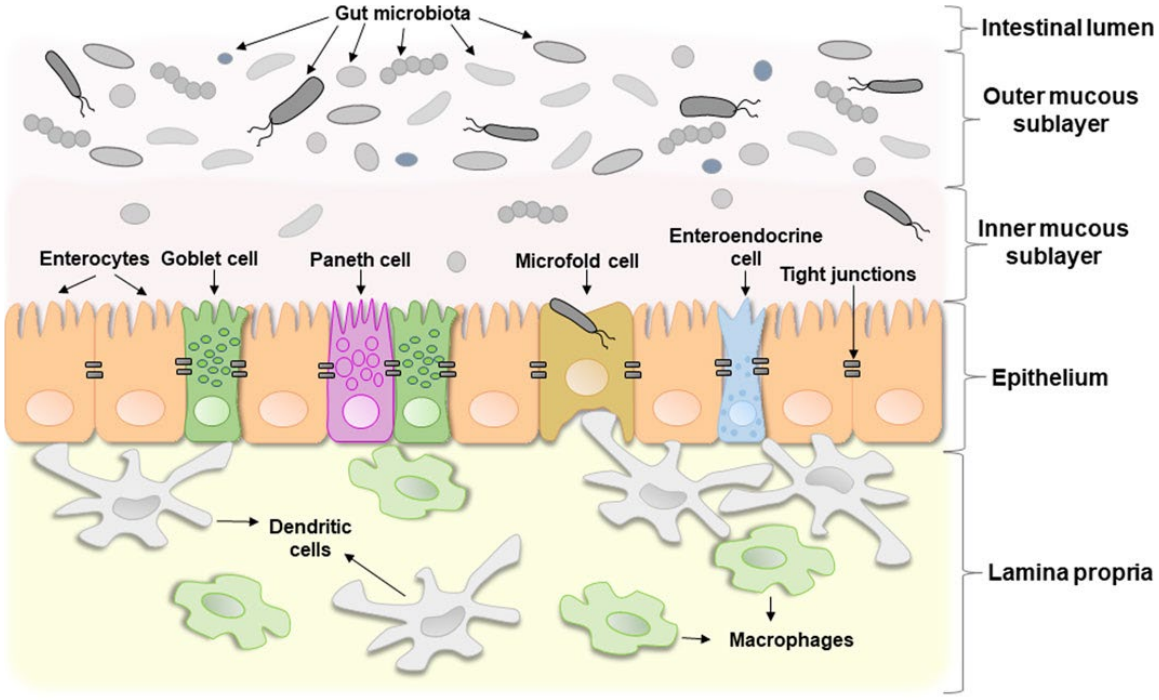

Figure 1.1 Composition and structure of small intestine and large intestine

The intestine consists of multiple specialized layers, including the intestinal lumen, mucus layers (outer and inner mucus sublayers), epithelium, and lamina propria. The intestinal epithelium is composed of various cell types, including enterocytes, which absorb nutrients; goblet cells, which secrete mucins to form a protective barrier; Paneth cells, which produce antimicrobial peptides; microfold (M) cells, which facilitate antigen uptake; and enteroendocrine cells, which regulate gut function through hormone secretion. Additionally, immune cells such as dendritic cells and macrophages reside in the lamina propria, contributing to immune surveillance and maintaining intestinal homeostasis. These structures work together to form a complex intestinal barrier that enables both nutrient absorption and immune defense. This image is based on Dmytriv et al, 2024. Frontiers in Physiology © open access article distributed under the Creative Commons

1.2.2 Physiological function of the intestine in humans and animals

The gastrointestinal tract is a core of human and animal physiology and health, playing a crucial role in nutrient digestion, absorption and secretion as well as protecting against pathogenic agents or unwanted microbial and viral infections (Motta et al., 2021). Furthermore, the intestine has the unique regenerative property after any damage caused by infections, radiation, or other influences (Hageman et al., 2020). The body has a very sophisticated structure and physiology which are extremely adjusted to carry out these vital functions that regulate tissue growth and maintain a state of homeostasis in both humans and animals.

1.2.2.1 Digestive and absorptive functions

The main function of the gastrointestinal tract is digestion and absorption, which are the basic processes for the small and large intestine (Bany Bakar et al., 2023). The small intestine is provided with finger-like projections (villi) and microvilli that broaden the intestinal area to maximize the absorption of nutrients (Harnik et al., 2024). These structural adaptations allow the small intestine to efficiently absorb digested carbohydrates, proteins, and fats, which are first broken down by pancreatic enzymes and bile acids before being taken up by enterocytes (Yang et al., 2020). The small intestine is divided into three main sections: duodenum, jejunum, and ileum; each section plays a specific role in nutrient absorption (Zwick et al., 2024). In the duodenum, bile from the liver and digestive enzymes from the pancreas are released to begin the breakdown of fats, proteins, and carbohydrates (Ko et al., 2020). Jejunum is the main site for the absorption of most nutrients, including amino acids, monosaccharides, and fatty acids (Muramatsu and Winter, 2024). Then, the

ileum absorbs vitamins, such as vitamin B12, and reabsorbs 95% bile salts (Girard et al., 2001; Deng and Bae, 2020). The large intestine involves the cecum, colon, and rectum. Its primary function is to absorb water and electrolytes from undigested food matter by osmosis and diffusion (Blachier et al., 2020). By compacting, it provides a way for solid feces to be formed, preventing dehydration by helping the body maintain fluid balance. Some studies have indicated that the colon is a rather effective organ in water and ion reabsorption, although different parts of it perform different functions (Phillips, 1969). For example, water absorption is primarily done by the ascending portion of the colon, while the descending portion continues when the stool accumulates (Jensen et al., 2023). Besides, a complex and dense community of microorganisms is housed within the large intestine, commonly referred to as the gut microbiota. Those bacteria ferment undigested carbohydrates, producing SCFAs such as butyrate, acetate, and propionate. These SCFAs serve as the primary energy source for colonic cells and positively impact their health (Nogal et al., 2021). Fermentation of food in the large intestine also contributes to the production of various vitamins, including vitamin K, which is important for blood clotting, and B vitamins (Daisley et al., 2021).

Absorption of nutrients in the small intestine starts with enzymatic processes that break down large molecules into smaller ones for further absorption. Carbohydrates are degraded into monosaccharides via the hydrolysis by particular enzymes, such as amylase, while proteins are severed into amino acids by proteases, such as trypsin and chymotrypsin (Gray, 1992; Gurumallesh et al., 2019) (Figure 1.2 A, B). Fats are emulsified by bile acids and then broken down into fatty acids and glycerol by lipase (Xu et al., 2021a) (Figure 1.2 C).

The degradation of the macronutrients into smaller molecules is followed by absorption into the enterocytes via various mechanisms depending on the type of nutrients, including passive diffusion, active transport, and endocytosis. The efficiency of digestion and nutrient absorption in the small intestine relies on its structure. It is also influenced by the movements of the gastrointestinal tract as well as the secretion of digestive enzymes (Mackie et al., 2020). The contribution of hormones, such as cholecystokinin and gastrin, in stimulating the secretion of bile and pancreatic enzymes is well-documented. These hormones also play a key role in facilitating and coordinating intestinal muscle movements, ensuring smooth and orderly transit of food through the gastrointestinal tract (Woźniak et al., 2021). Additionally, the intestinal crypts of Lieberkühn, located at the base of the villi, continuously generate new enterocytes to replace older or damaged cells, preserving the integrity and function of the intestine (Hohman and Osborne, 2022).

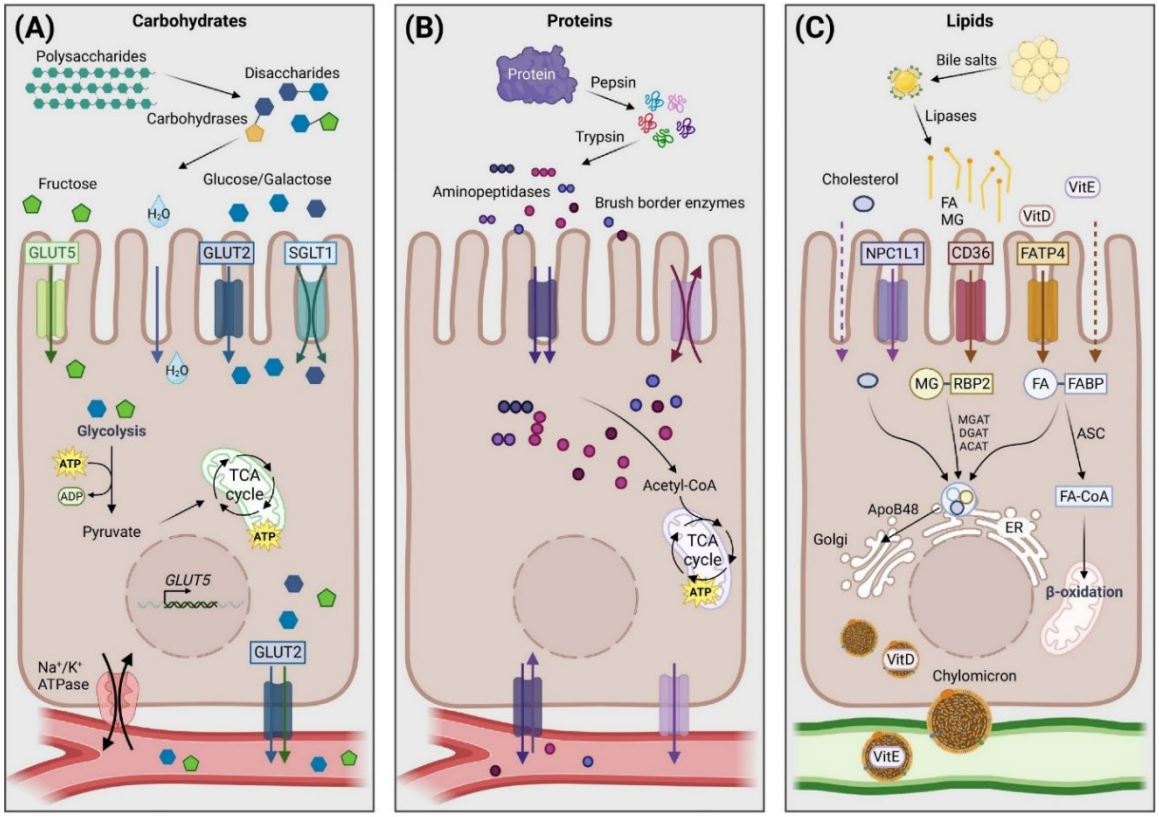

Figure 1.2 Gut epithelial metabolism

(A) Carbohydrates are broken down into monosaccharides, such as glucose, galactose, and fructose, through enzymatic hydrolysis by carbohydrase. These monosaccharides are then transported into enterocytes via specific transporters, including glucose transporter 5 (GLUT5) for fructose and sodium-glucose linked transporter 1 (SGLT1) for glucose and galactose, followed by glucose transporter 2 (GLUT2)-mediated transport into the bloodstream. (B) Proteins are degraded into peptides and amino acids by proteases, such as pepsin and trypsin. Brush border enzymes further hydrolyze peptides into amino acids, which are absorbed through various amino acid transporters and utilized in metabolic pathways like the TCA cycle. (C) Lipids undergo emulsification by bile salts and hydrolysis by lipases, yielding free fatty acids (FA), monoglycerides (MG), and cholesterol. These molecules are absorbed by enterocytes via transporters such as Niemann-Pick C1-

like 1 (NPC1L1) for cholesterol and cluster of differentiation 36 (CD36) / fatty acid transport protein 4 (FATP4) for fatty acids. Once inside, they are re-esterified and packaged into chylomicrons for transport via the lymphatic system. This image is based on Schwärzler et al, 2024. Trends in Cell Biology

1.2.2.2 Intestinal barrier function

Another important role of the intestine is to act as a selective barrier, which controls the entry and exit of substances by allowing or blocking their passage, thereby determining which substances enter or leave the body (Paone and Cani, 2020). Indeed, the integrity and function of this barrier are essential to gut health. This multi-layered barrier allows nutrients and electrolytes to enter the bloodstream while preventing the harmful pathogens from entering the body (Régnier et al., 2021). The selective barrier is composed of several key components. In the lumen, digestive secretions such as pancreatic enzymes play an initial role in breaking down ingested bacteria and antigens (Sarowska et al., 2019). Commensal bacteria also contribute to the defense by producing antimicrobial substances that inhibit the colonization of pathogens (Buffie and Pamer, 2013). Additionally, close to the epithelium lies a critical protective zone consisting of the unstirred water layer, glycocalyx, and the mucus layer, which work together to prevent bacterial adhesion (Kraehenbuhl and Neutra, 2000). The mucus is produced by goblet cells, trapping harmful microorganisms and preventing their direct contact with the epithelial surface (Gustafsson and Johansson, 2022). Moreover, Paneth cells are located in the crypts of Lieberkühn and secrete antimicrobial peptides such as α-defensins and lysozymes, which help maintain microbial balance and neutralize pathogens (Jandl et al., 2024). Secretory immunoglobulin A (IgA), produced by plasma cells, also plays a crucial role in immune defense. It can bind pathogens and toxins to neutralize them (Pietrzak et al., 2020).

Another line of defense involves the intestinal epithelial layer, which consists of tightly connected cells that control what passes between the intestinal lumen and the body. Tight

junction proteins, including claudins, occludin, and ZO-1, seal these epithelial cells together, allowing for the selective movement of water, ions, and nutrients while harmful substances are denied entry (Miner-Williams and Moughan, 2016). However, these junctions can be disrupted by inflammation, infections, or oxidative stress, leading to increased intestinal permeability, a condition known as "leaky gut" (Yu et al., 2022a). This breakdown of the barrier function has been implicated in various gastrointestinal diseases, including inflammatory bowel disease and celiac disease (John et al., 2011; Luissint et al., 2016). The key layers of the intestinal barrier are shown in Figure 1.3.

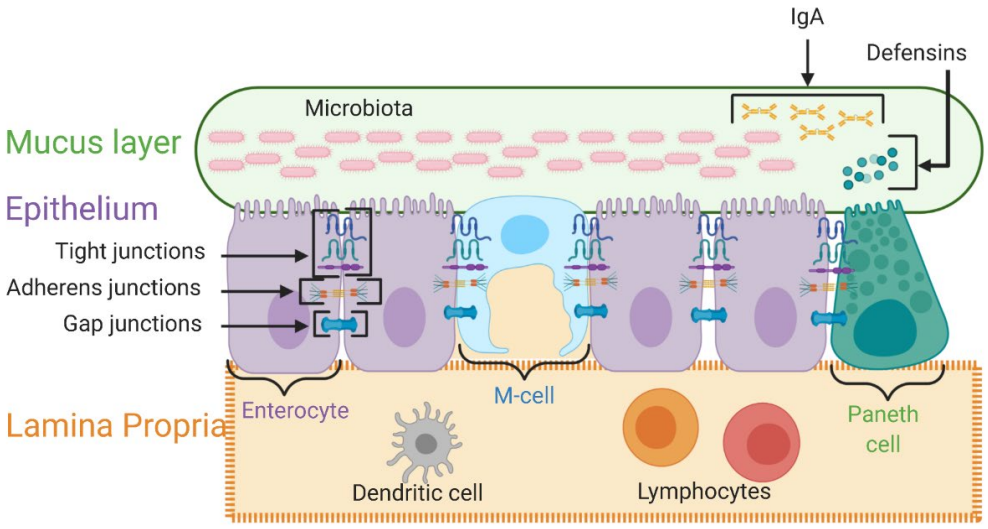

Figure 1.3 Key layers of the intestinal barrier

The intestinal barrier is composed of multiple layers, including the mucus layer, epithelial layer, and lamina propria, which work together to regulate selective permeability and immune defense. The mucus layer harbors the gut microbiota and contains immunoglobulin A (IgA) and defensins, which help prevent pathogen invasion. The epithelial layer consists of different cell types. Tight junctions, adherens junctions, and gap junctions maintain epithelial integrity by regulating intercellular permeability. The lamina propria contains immune cells such as dendritic cells and lymphocytes, which contribute to immune surveillance and response. This image is based on Sharma et al, 2020. Microorganisms © open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1.2.2.3 Immune function

The gastrointestinal tract is a central player in the body's immune system, primarily through the gut-associated lymphoid tissue (Figure 1.4). Gut-associated lymphoid tissue constantly monitors the intestinal lumen for harmful pathogens while maintaining tolerance to beneficial microbes and dietary antigens, preventing unnecessary immune reactions (Fehervari and Kiyono, 2008). A key component of gut-associated lymphoid tissue is Peyer's patches, which contain B cells, T cells, dendritic cells, and macrophages that maintain immune surveillance and trigger both humoral and cell-mediated immune responses (Jung et al., 2010; Da Silva et al., 2017). There are also some isolated lymphoid tissues in the large intestine, which are mainly composed of B cells (Mowat and Agace, 2014). Enterocytes provide defense through the secretion of secretory IgA, an immunoglobulin that acts via the neutralization of pathogenic microbes before they cross the epithelial barrier (Ramanan and Cadwell, 2016). Intestinal epithelial cells are also invested in intraepithelial lymphocytes and macrophages, both capable of producing pro-inflammatory cytokines like tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), and interleukin-1 beta (IL-1β). These cytokines play an important role in initiating the inflammatory response during infection or after tissue injury (Zganiacz et al., 2004; Licastro et al., 2005; Neurath, 2014). Paneth cells secrete antimicrobial peptides, such as defensins and lysozyme, which help regulate the gut microbiota and resist harmful pathogens (Bevins and Salzman, 2011). The gut microbiota also plays a critical role in regulating the immune response by producing SCFAs, which supports the function of T regulatory cells that limit excessive inflammation and maintain immune tolerance (Omenetti and Pizarro, 2015; Kim, 2021).

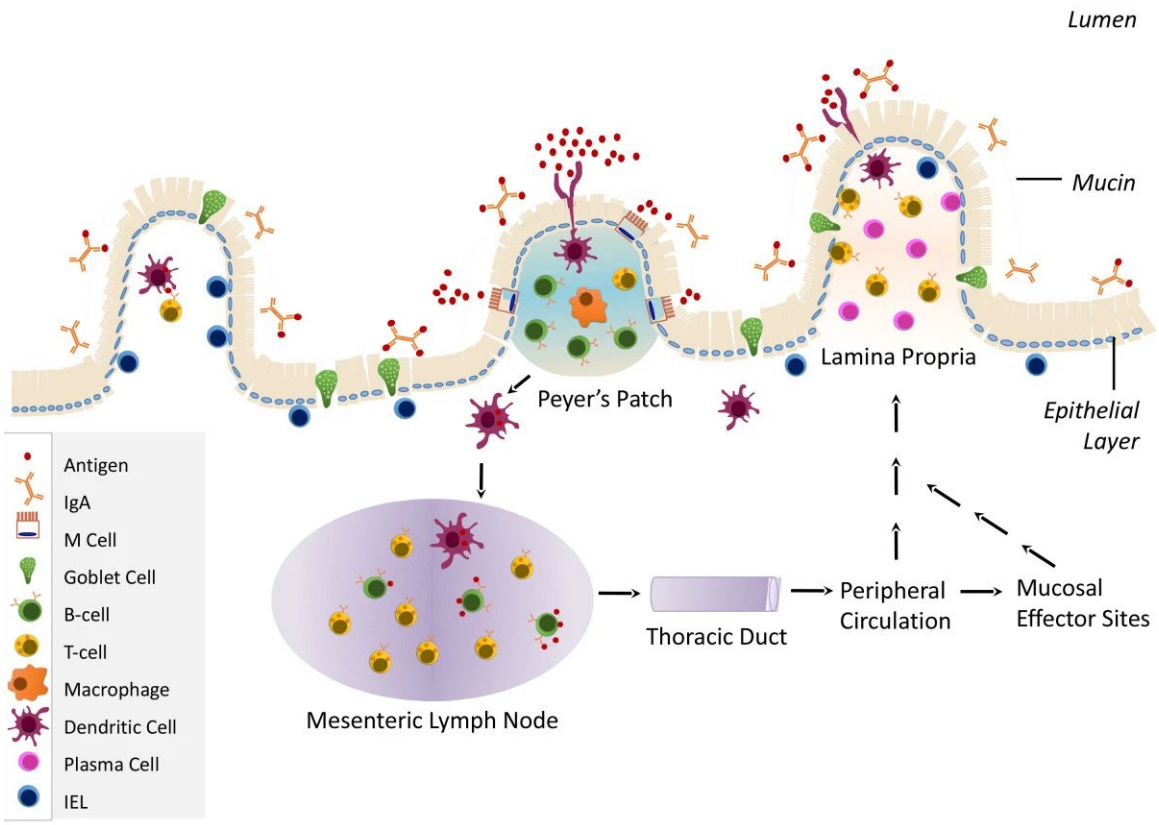

Figure 1.4 Gut-associated lymphoid tissue

The gastrointestinal tract is a key component of the body's immune system, with gut-associated lymphoid tissue serving as a primary site for immune surveillance and response. Antigens from the intestinal lumen are sampled by microfold (M) cells, which transport them to underlying immune cells in Peyer's patches, specialized lymphoid structures in the intestinal epithelium. Within the lamina propria, immune cells such as dendritic cells, macrophages, B cells, T cells, and plasma cells coordinate immune responses. Secretory immunoglobulin A (IgA), produced by plasma cells, plays a crucial role in neutralizing pathogens. The activation of immune cells in gut-associated lymphoid tissue leads to antigen processing and migration to mesenteric lymph nodes, where immune responses are further orchestrated. Activated immune cells then enter the thoracic duct and circulate through the peripheral circulation, ultimately migrate back to mucosal effector sites to

maintain gut immune homeostasis. This image is based on Ruth et al, 2013. Journal of Animal Science and Biotechnology

1.2.2.4 Secretory function

Intestinal secretory function is critical in the dissolution and dilution of nutrients for their active transport, maintenance of gut hydration, and promotion of smooth intestinal mobility (Dolan et al., 2022; Gustafsson and Johansson, 2022). Goblet cells synthesize mucus, which is a physical barrier protecting the epithelium and enabling the smooth passage of gut contents (Johansson et al., 2011; Gustafsson and Johansson, 2022). Enterocytes secrete water and electrolytes to create an environment favorable for digestion and absorption (Wapnir and Teichberg, 2002). All these put together maintain intestinal homeostasis to support efficient functioning of the gastrointestinal tract. Secretion in the intestine depends on the movement of water and electrolytes into the lumen through osmotic gradients mediated by specific transport mechanisms involving the net movement of sodium coupled to the secretion of proton secretion, and/or solute-coupled water transport (Ruhr et al., 2014). However, disruption caused by toxins, infection, or drugs may lead to excessive secretion manifesting as diarrhea or, less frequently, as dehydrating states (Bearcroft et al., 1996; Koyyada, 2021; Kwiatkowska et al., 2021; van Hoffen et al., 2021; Biernbaum and Kudva, 2022). Neuroendocrine factors such as serotonin, vasoactive intestinal peptide, and substance P stimulate fluid secretion (McFadden et al., 1986; Grishina et al., 1998; Arcuni et al., 2000). Bacterial toxins, such as those from *E. coli* and *Vibrio cholerae*, activate chloride secretory pathways via cyclic adenosine monophosphate and cyclic guanosine monophosphate signaling with the subsequent outflow of sodium and fluid (Ramamurthy et al., 2020; Ye et al., 2024). The intestine also possesses mechanisms for opposing excessive secretion. Nutrients in the lumen stimulate the release of pro-absorptive hormones, which reduce fluid loss and restore balance (Asadpoor et al., 2021). Hormones

like neuropeptide Y and antisecretory factors play a role in this regulation (Anthone et al., 1991; Johansson et al., 1997). Nitric oxide may act as a second messenger and, thus, influence the secretory-absorptive balance by affecting intracellular signaling pathways (Stoner et al., 2000). This complex regulatory system ensures that intestinal secretory processes support digestion while protecting against over-secretion and maintaining fluid and electrolyte homeostasis.

1.3 Factors affecting intestinal health

Intestinal health is critical for the overall growth, immunity, and performance of animals. A well-functioning intestine ensures optimal nutrient absorption, provides a barrier against pathogens, and supports immune responses. A variety of factors can disrupt the integrity and function of the intestinal system (Stoner et al., 2000). Changes in diet, the microbiome, the environment and an increase in stressors have been shown to impair intestinal health. These factors are often exacerbated during critical transition periods, such as the weaning period in piglets.

1.3.1 Weaning stress

Weaning is considered a critical and stressful period in the early life of piglets, during which there is a sudden transition from milk nutrition to solid feed. This transition is further intensified by separation from the sow and exposure to new environmental and social conditions. These changes result in significant dietary, environmental, and psycho-emotional stress, affecting the structural and functional integrity of the gastrointestinal tract in piglets (Moeser et al., 2017). The rapid changes during weaning lead to a phenomenon

commonly referred to as weaning stress. Some studies have shown that weaning stress causes a decrease in villus height and the villus-to-crypt ratio as well as an increase in crypt depth (Wang et al., 2022b). These changes indicate impaired absorption capacity and intestinal health, which reduces the efficiency of nutrient absorption. Also, such a dramatic reduction in villus surface area seriously diminishes the capacity of the small intestine to handle the new solid food and might result in malabsorption with further stress on the digestive system (Stojanović et al., 2021). Furthermore, weaning is a process closely linked with impaired intestinal barrier function (Degroote et al., 2020; Stojanović et al., 2021). The intestinal epithelium normally serves as a protective barrier to pathogens. The impaired intestinal epithelium can lead to increased intestinal permeability (Akdis, 2021). This provides better opportunities for pathogenic bacteria such as ETEC to colonize the intestine, contributing to post-weaning diarrhea (Kim et al., 2022b). Disruption or reduction in tight junction proteins like ZO-1 and claudin-1 further increases intestinal permeability, permitting the translocation of toxins and harmful microorganisms into the blood, which can result in systemic infections (Ghosh et al., 2020; Li et al., 2020b). Along with physical barrier dysfunction and increased membrane permeability, weaning stress also promotes oxidative stress and inflammation, causing intestinal damage (Tang et al., 2022). In addition, weaning stress elevates ROS production, which overwhelms the gastrointestinal antioxidant defense and results in lipid peroxidation and protein oxidation of intestinal cells (Hussain et al., 2021). This leads to oxidative damage to the epithelial integrity, leading to intestinal permeability and inflammation, which alters gut function (Hussain et al., 2021). At this stage, higher levels of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β are

observed, promoting inflammation and intestinal dysfunction (Liu et al., 2024b). The negative impacts of weaning stress on swine gut health are shown in Figure 1.5.

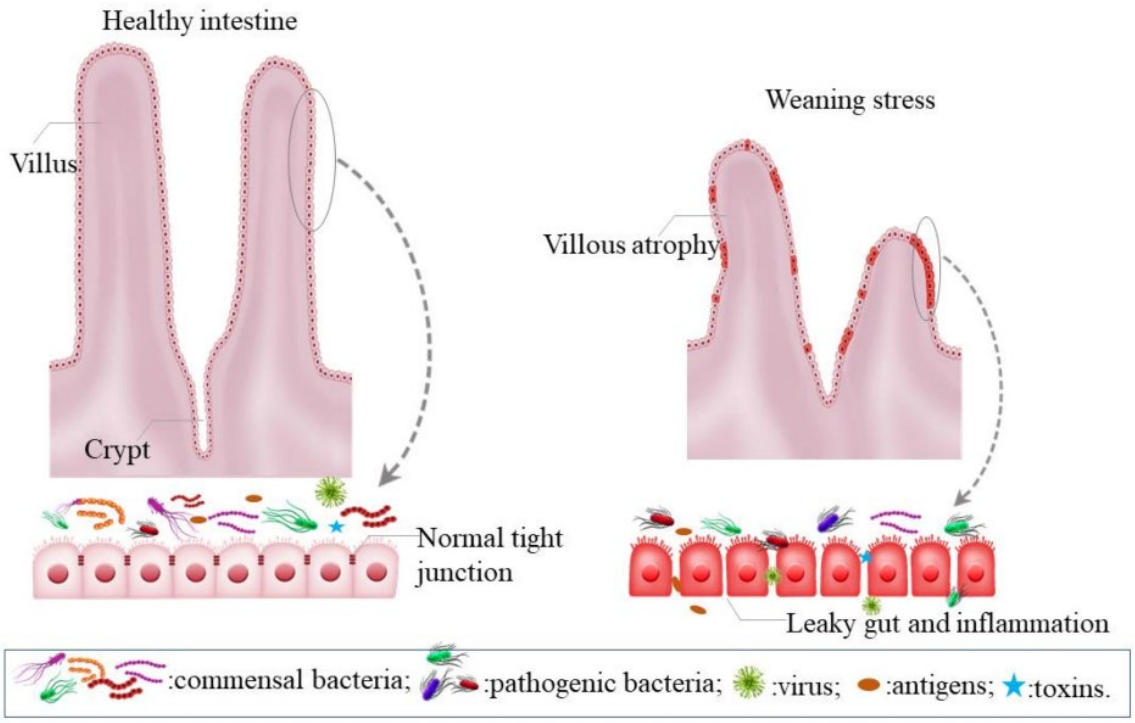

Figure 1.5 Negative impacts of weaning stress on swine gut health

The left panel illustrates a healthy intestine, characterized by well-structured villi and crypts, intact epithelial cells, and normal tight junctions, which maintain gut barrier integrity. The microbial community consists of a balanced population of commensal bacteria, helping to regulate gut homeostasis. The right panel illustrates the effects of weaning stress, which induces villous atrophy, disrupts tight junctions, and increases intestinal permeability, leading to a condition known as leaky gut. This disruption allows for the translocation of pathogenic bacteria, viruses, antigens, and toxins, triggering intestinal inflammation. This image is based on Wei et al, 2024. Animals © open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

1.3.2 Dietary factors

Diet is one of the main factors regulating gut microbiota. Diet changes, such as weaned piglets switching from a milk diet to solid feed, can impair intestinal health and growth performance and increase disease susceptibility. This shift dramatically impacts the gut microbiome, affecting the digestion and absorption of nutrients in the intestine (Luo et al., 2022b). Dietary fiber is critical for maintaining intestinal health by serving as a substrate for microbial fermentation (Qin et al., 2021). Fermentation can produce SCFAs such as butyrate, acetate and propionic acid (Hays et al., 2024). Among these, butyrate is particularly important for promoting epithelial cell proliferation, maintaining mucosal integrity, and supporting immune function (Lu et al., 2022). Also, butyrate maintains gut anaerobiosis and is essential for the growth of favorable bacteria and the prevention of pathogens, like *Escherichia coli* (*E. coli*) (Siddiqui and Cresci, 2021). A recent study has shown that gut protection can be enhanced with adequate amounts of fiber in the diet (Zou et al., 2018). Under this condition, the growth of pathogenic bacteria is inhibited, and beneficial gut microbes (such as *Lactobacillus* and *Bifidobacterium*) are nourished. These beneficial gut microbes are participating in maintaining intestinal mucosal protection and immune function (Bested et al., 2013; Dong et al., 2022). Some studies have shown that insoluble fibers can elevate levels of SCFAs and improve the intestinal mucosal barrier function, leading to a reduced incidence of post-weaning diarrhea (Chen et al., 2019, 2020; Uddin et al., 2023). Similarly, soluble fibers, such as oligosaccharides, promote SCFAs production, enhance gut barrier function, lower intestinal pH, and inhibit pathogenic bacteria (Zhang et al., 2022a). The positive effects of dietary fiber on swine gut health are illustrated in Figure 1.6.

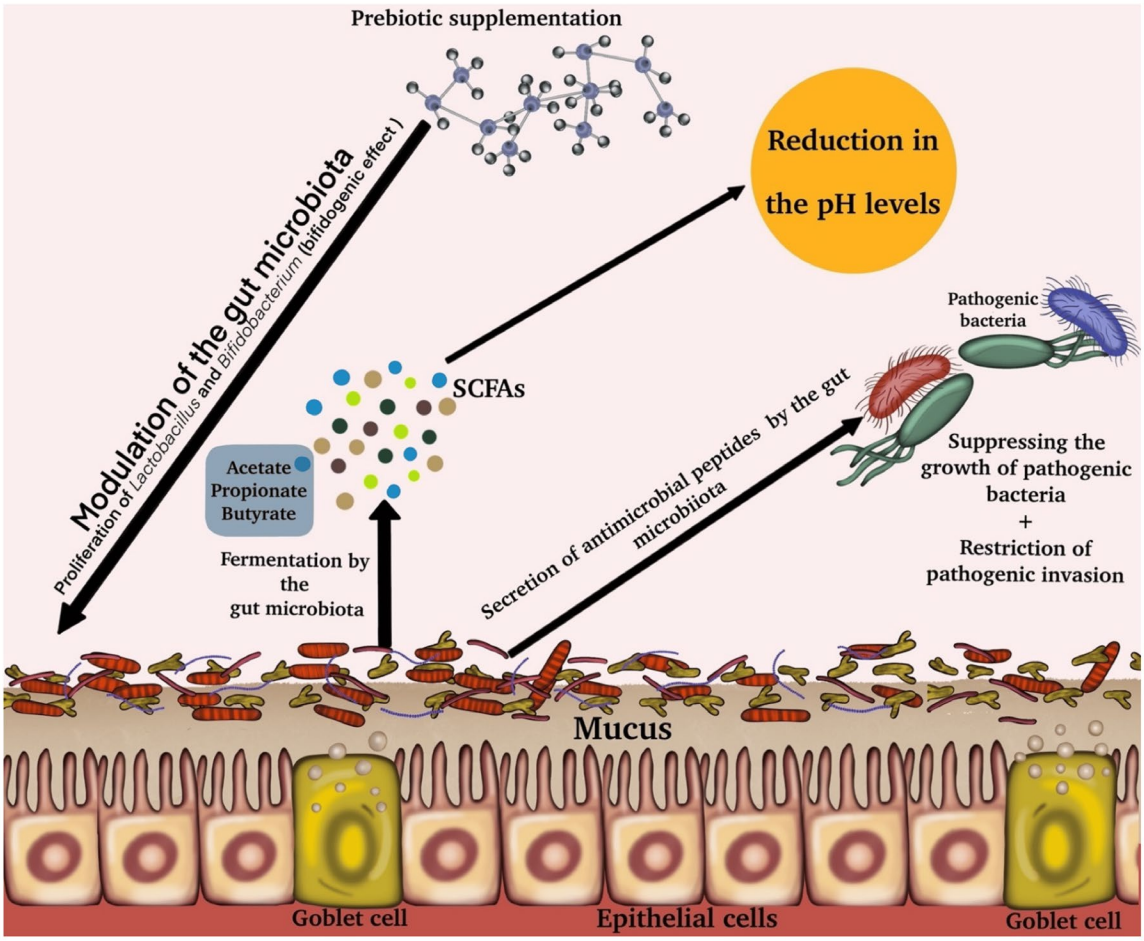

Figure 1.6 The positive impacts of dietary fiber on swine gut health

Prebiotic supplementation, primarily in the form of dietary fiber, promotes the modulation of gut microbiota, particularly by stimulating the proliferation of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* while inhibiting harmful bacteria. Fermentation of dietary fiber by gut microbiota leads to the production of short-chain fatty acids (SCFAs), including acetate, propionate, and butyrate, which provide multiple benefits. SCFAs contribute to pH reduction in the gut, creating an environment that limits the growth of pathogenic bacteria. Additionally, SCFAs enhance the secretion of antimicrobial peptides by the gut microbiota, further suppressing the proliferation and invasion of pathogens. This image is based on Megur et al, 2022. International Journal of Molecular Sciences © open

On the contrary, diets containing high-fat and low-fiber were associated with gut dysbiosis, a condition characterized by an imbalance in gut microbiota (Bailén et al., 2020). Dysbiosis can result in increased inflammation, reduced microbial diversity, and a higher risk of post-weaning diarrhea (Han et al., 2023). A recent study has shown that insufficient dietary fiber in the diets of weaned piglets impairs intestinal health (Hu et al., 2023). Restoration of the microbial composition and gut homeostasis against these effects is the aim of dietary management, such as prebiotics and probiotics. Oligosaccharides, primarily fructo-oligosaccharides and galacto-oligosaccharides serve as prebiotics, enhancing probiotic-mediated benefits and regulating intestinal barrier and immune reactions (Hu et al., 2024). Similarly, probiotic *Lactobacillus plantarum* has been identified to reduce inflammation, prevent harmful bacteria from colonizing, and promote SCFAs production (Yue et al., 2020).

1.3.3 Infectious factors

Intestinal health is easily affected by infections, especially in environments with poor hygiene. Some studies have shown that poor hygiene conditions can encourage the growth of harmful bacteria (including *E. coli* and *Salmonella*), increasing the risk of intestinal infection with harmful bacteria (Vukmirović et al., 2017; Wang et al., 2021; Soliani et al., 2023). Enterotoxigenic *Escherichia coli* has been described as the most common diarrheagenic organism isolated from post-weaning piglets suffering from diarrhea. ETEC F4 and ETEC F18 are the most well-known in pig production, both of which can express specific fimbriae. These fimbriae bind to specific receptors on intestinal epithelial cells, allowing ETEC to colonize the intestine and cause infection (Kim et al., 2022b; von

Mentzer and Svennerholm, 2024). ETEC infection also results in economic losses due to reduced growth performance and increased morbidity and mortality (Wu et al., 2021b). Once ETEC has adhered to the intestinal epithelium, it secretes a variety of enterotoxins, the most significant being heat-labile toxin and heat-stable toxins (STa and STb) (Zhou et al., 2021a). These toxins disrupt electrolyte balance, leading to the hypersecretion of chloride ions and water into the intestinal lumen, resulting in watery diarrhea (Yamamoto et al., 2007). Labile toxin exerts its effects by activating adenylate cyclase, increasing intracellular cyclic adenosine monophosphate, which in turn triggers chloride and bicarbonate ion secretion while inhibiting sodium absorption (Keely and Barrett, 2022). This leads to massive fluid loss, dehydration, and potentially life-threatening diarrhea. Heat-stable toxin acts through guanylate cyclase, increasing cyclic guanosine monophosphate levels, which similarly causes chloride and water secretion (Lima and Fonteles, 2014). This toxin also exacerbates intestinal permeability by promoting the release of inflammatory cytokines such as IL-6 and interleukin-8, further disrupting the tight junction integrity of the intestinal epithelium. Additionally, Heat-stable toxin b interacts with calcium channels on enterocytes, leading to elevated intracellular calcium levels, contributing to increased paracellular permeability and fluid loss (Nassour and Dubreuil, 2014).

Maintaining a strong gut barrier is essential for gut health, which relies on the epithelial cell layer sealed by tight junctions. ETEC severely damages this barrier, increasing intestinal permeability (Yi et al., 2021). It disrupts the expression and distribution of tight junction proteins like occludin, claudins, and ZO-1 (Yang et al., 2014). During infection,

ETEC produces heat-labile and heat-stable toxins (STa and STb) that target epithelial cells, impairing tight junction function (Ngendahayo Mukiza and Dubreuil, 2013). This disruption weakens the barrier, increasing paracellular permeability and facilitating the passage of pathogens and toxins through the epithelial layer. The broken intestinal barrier can also result in bacterial translocation, where bacteria or their components invade normally sterile tissues, such as the mesenteric lymph nodes, liver, and spleen (Rogers et al., 2023). Previous studies have shown that tight junction protein loss is closely associated with inflammatory pathway activation, increasing the expression of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-1β. The inflammatory responses further weaken the tight junctions (Ji et al., 2013). This cytokine-mediated disruption enhances the paracellular movement of water, electrolytes, and harmful pathogens across the epithelium (Perdue and McKay, 1994). Studies have also demonstrated that during ETEC infection, the villus height is reduced, and the crypt depth increases, which further impairs nutrient absorption and exacerbates the severity of diarrhea (Yang et al., 2014). ETEC infection also activates the innate immune response in the gut, attracting neutrophils and macrophages to the infection site (Noel et al., 2018). These immune cells produce reactive oxygen species and pro-inflammatory cytokines, leading to oxidative stress and damaging tight junctions (Yang et al., 2013). Additionally, lipopolysaccharides from ETEC bind to epithelial receptors, triggering inflammatory cascades that degrade the epithelial barrier (Finamore et al., 2014). This disruption increases diarrhea risk and systemic infections as bacteria and toxins enter the bloodstream. The impacts of ETEC on swine gut health are shown in Figure 1.7.

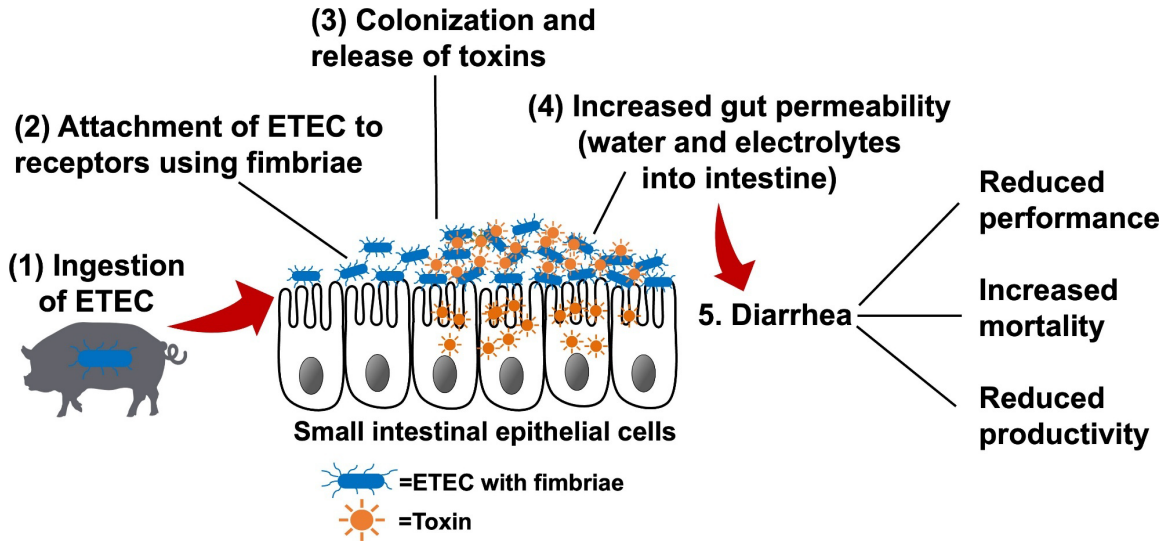

Figure 1.7 The impacts of enterotoxigenic *Escherichia coli* (ETEC) on swine gut health

The infection begins with the ingestion of ETEC (Step 1), followed by attachment of ETEC to epithelial receptors via fimbriae (Step 2). Once attached, ETEC colonizes the small intestinal epithelium and releases enterotoxins (Step 3), which disrupt the intestinal barrier, leading to increased gut permeability and excessive secretion of water and electrolytes into the intestinal lumen (Step 4). This results in severe diarrhoea (Step 5), which negatively affects pig health and performance, contributing to reduced productivity, increased mortality, and impaired growth performance. This image is based on Kim et al, 2022. Frontiers in Immunology © open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Other pathogens also have a severe impact on pig intestinal health. Some studies have shown that bacteria such as *Salmonella spp.*, *Clostridium difficile*, *Lawsonia intracellularis*, and *Brachyspira hyodysenteriae* disrupt gut function by colonizing the intestines, impairing the mucosal barrier, and increasing inflammation (Patel and McCormick, 2014; Sánchez de Medina et al., 2014; Vannucci and Gebhart, 2014; Quintana-Hayashi Macarena et al., 2017). Among these, *Salmonella* spp. is an occasional source of enteritis and systemic infections and *Clostridium difficile* causes severe colitis, diarrhea, and tissue damage. Viruses also contribute to intestinal damage in pigs. Viruses like porcine reproductive and respiratory syndrome virus, porcine epidemic diarrhea virus, and rotavirus impair the intestinal epithelium cells, inhibit nutrient absorption, and increase inflammation (Wang et al., 2019b; Amimo et al., 2021; Zhao et al., 2021). Parasites, including *Ascaris suum* and *Isospora suis*, can also damage the intestinal epithelium cells (Worliczek et al., 2009; Koehler et al., 2021). This disruption leads to poor nutrient absorption and diarrhea, exacerbating the overall health challenges faced by infected pigs.

1.4 Intestinal health in swine

Maintaining a healthy intestine has always been a key aspect of livestock production. A healthy intestine not only provides effective digestion and nutrient absorption, but also supports the immune defense and overall growth performance of the pigs. A well-functioning intestine is critical to improving animal welfare, physiological and mental wellness, and growth performance (Celi et al., 2017). Moreover, the intestinal system of pigs is complex and relatively stable and is highly susceptible to numerous factors, resulting in huge economic losses (Tang et al., 2022). The growth of pigs is commonly

divided into piglet and growing-finishing stages, each characterized by distinct intestinal developmental profiles and functional requirements.

1.4.1 Piglets

Intestinal development at the piglet stage is essential to support the rapid growth and overall health of the pig. After birth, piglets undergo rapid growth and significant intestinal development, including increased villus height, providing a stronger structural basis for efficient nutrient absorption. (Li et al., 2022b). In this early life stage, maternal milk delivers vital nutrients and key immune factors (immunoglobulins) to set up an early intestinal barrier and minimize pathogen invasion (Li et al., 2022a). As the diet switches from milk to solid feed and the environment and psychology become challenging, the piglet's intestine suffers significant stress after weaning (Tang et al., 2021). A significantly observed alteration in the intestinal structure during weaning is the villus height decreasing and crypt depth increasing, which results in decreased absorptive capacity and increased gut permeability (Shi et al., 2022). These changes make piglets susceptible to gastrointestinal health issues, such as diarrhea (Su et al., 2022a). Therefore, a healthy intestine during the weaning period typically exhibits relatively high villus height and shallow crypt depth to support optimal nutrient absorption.

1.4.2 Growing-finishing pigs

The pig's intestine gradually adapts to more complex and varied solid feed in the growing-finishing stage. Compared to piglets, the intestinal structure in growing pigs is more mature, with increased small intestine length and significantly expanded surface area to meet the

high energy and protein intake required for rapid growth (Pluske, 2016). Meanwhile, digestive enzyme activity increases further, enabling the intestine to efficiently process complex nutrients from solid feed. At this stage, the criteria for evaluating intestinal health focus on digestive and absorptive efficiency, stability of the villi and crypts, and integrity of the gut barrier. Villus height and crypt depth remain stable in growing pigs, ensuring continuous and efficient nutrient absorption. Meanwhile, the integrity of tight junction proteins maintains an effective gut barrier, preventing pathogens and toxins from entering the bloodstream. Growing pigs also exhibit a stronger adaptive capacity to the changes in diet and environment, which is beneficial for maintaining a healthy intestinal environment in high-density farming conditions (Jha and Berrocoso, 2015). The significant differences in intestinal structure and function between growing pigs and piglets reflect their changing nutritional requirements. Piglets rely on the nutrients and immune components in maternal milk to support the initial gut barrier and immune function, whereas growing pigs rely on their developed digestive enzyme systems and stable gut barrier to ensure efficient nutrient utilization and a healthy growth environment.

1.5 Oxidative stress and gut health

Oxidative stress refers to an imbalance between the production of ROS and the body's ability to detoxify these reactive radicals (Sies and Jones, 2020) (Figure 1.8). Under normal conditions, ROS play essential roles in cellular signaling and defense mechanisms (Sies and Jones, 2020). However, when ROS are produced in excess, these free radicals can damage the antioxidant defense system, leading to modification of proteins, lipids and DNA within cells, thereby affecting cell function and viability (García-Sánchez et al.,

2020). For pigs, regulation of oxidative stress is crucial for maintaining gut health, optimizing growth, and ensuring resilience against various environmental stressors.

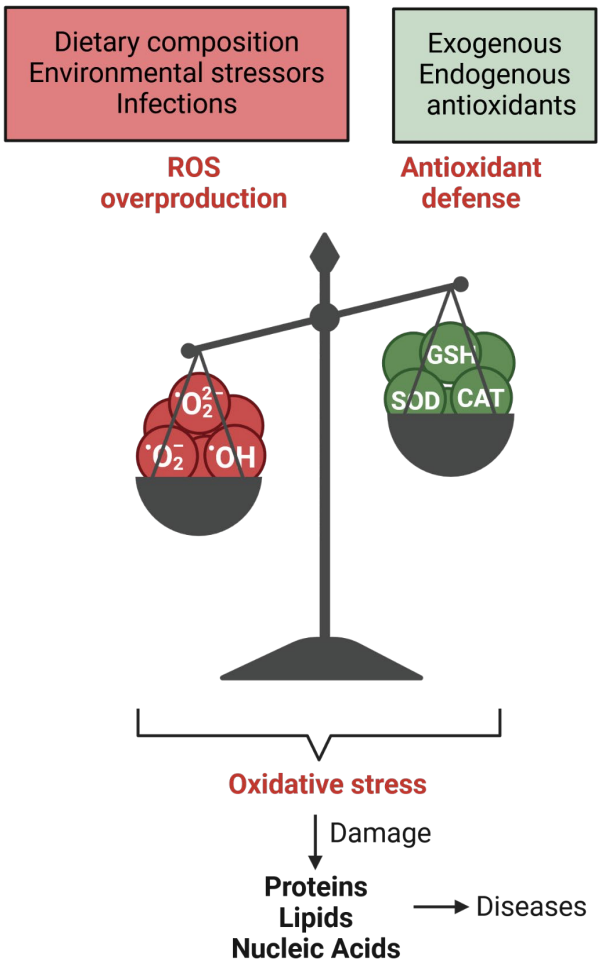

Figure 1.8 Redox homeostasis

Oxidative stress arises when there is an imbalance between ROS overproduction and the body's antioxidant defenses. Factors such as dietary composition, environmental stressors, and infections contribute to excessive ROS generation, including superoxide anion ($O_2^{-}\bullet$), hydrogen peroxide (H_2O_2), and hydroxyl radicals (•OH). To counteract oxidative damage, the body relies on endogenous and exogenous antioxidants, including superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). When ROS levels exceed antioxidant capacity, oxidative stress leads to damage of proteins, lipids, and nucleic acids, ultimately contributing to the development of various diseases.

1.5.1 Impact of oxidative stress on intestinal health

Oxidative stress is well recognized as a damage mechanism within the gastrointestinal tract, because of the gastrointestinal tract's key role as the major barrier to ingested antigens, pathogens, and environmental toxins (Vona et al., 2021). High concentrations of reactive oxygen species can lead to severe damage to the cell, including lipid peroxidation, protein breakdown, and even DNA damage in the epithelial cells of the intestine (García-Sánchez et al., 2020). This leads to intestinal barrier impairment and increased permeability, enabling the leakage of pathogens and toxins and increasing the risk of systemic infection. Reactive oxygen species also target tight junction proteins (occludin and ZO-1), which are key in maintaining selective gut permeability (Paradis et al., 2021). Damage to these proteins results in a loss of barrier function, allowing endotoxins and microbes to translocate into the bloodstream, driving inflammation and even gut-related inflammatory diseases such as inflammatory bowel disease (Ghosh et al., 2021). In addition, oxidative stress alters mucus and immune barriers leading to impairments in nutrient absorption and intestinal health (Wang et al., 2020b).

Enterocytes, the main absorptive cells of the intestinal lining, are also susceptible to oxidative stress (Rath and Haller, 2022). Very high ROS levels can cause enterocyte apoptosis, leading to epithelial integrity loss and worsening intestinal barrier function (Sundaram et al., 2019; Xu et al., 2021b). In addition, oxidative stress can reduce the function and expression of important transport proteins, thereby affecting the absorption of nutrients. These proteins mainly include specialized proteins that help transport nutrients (glucose, amino acids and lipids) across cell membranes (Rummel and Butterfield, 2021;

Kotlyarov and Kotlyarova, 2022; Tang and Xiong, 2022). Under oxidative stress conditions, downregulation of these transporters can lead to reduced nutrient absorption efficiency and, consequently, nutrient deficiencies. Such nutrient deficiencies are particularly severe during periods of rapid growth, as they can prevent optimal health.

The effects of oxidative stress extend to inflammatory signaling pathways, where persistent oxidative imbalance triggers a chronic low-grade inflammatory response (Raut and Khullar, 2023). This process attracts immune cells, such as macrophages and neutrophils, to the site of damage. These cells release pro-inflammatory cytokines, including TNF-α and IL-6, which further compromise tight junction proteins and worsen epithelial barrier dysfunction. A cyclical relationship between oxidative stress, inflammation, and increased gut permeability leads to chronic nutrient malabsorption and contributes to conditions similar to inflammatory bowel disease due to prolonged immune activation (Tian et al., 2017).

1.5.2 Common factors causing oxidative stress in the gut

In livestock production, numerous factors contribute to oxidative stress in the gastrointestinal tract. Dietary composition plays a primary role, as diets high in fats and low in fiber are known to increase ROS production significantly (Ye et al., 2022). High-fat diets, especially those containing polyunsaturated fats, promote lipid peroxidation, resulting in harmful lipid peroxides that can damage enterocytes (Rohr et al., 2020). This effect is exacerbated by inadequate fiber intake, which limits the production of SCFAs like butyrate (Khoshbin and Camilleri, 2020). SCFAs have essential anti-inflammatory and

antioxidative properties that help reinforce gut barrier integrity and maintain redox balance (Huang et al., 2021). When SCFAs production is insufficient, as is often the case in animals fed processed and low-fiber feeds, the gut's protective capacity is compromised, allowing ROS accumulation and contributing to gut permeability and inflammation (Tan et al., 2023).

Environmental stressors also significantly contribute to oxidative stress, particularly in high-density farming systems. Heat stress is a widespread issue, as temperature fluctuations in densely populated environments activate the hypothalamic-pituitary-adrenal axis, releasing stress hormones that elevate ROS production (Goel et al., 2021). This ROS surge impairs immune function and damages the gut epithelium, reducing its ability to absorb nutrients effectively (Yun et al., 2020).

Pathogen exposure is another critical factor in oxidative stress within the gut. Enteric pathogens, such as *E. coli* and *Salmonella*, produce endotoxins that invade the gut lining and trigger an inflammatory response, significantly increasing ROS production (Chanin et al., 2020; Xia et al., 2020). Mycotoxins, toxic compounds produced by fungi in contaminated feed, further exacerbate oxidative damage. Aflatoxins and fumonisins, commonly found in low-quality feed, interfere with antioxidant defense by depleting glutathione levels and impairing antioxidant enzyme function, thus weakening the gut's capacity to counteract oxidative stress (Chen et al., 2021b; Mavrommatis et al., 2021). The combination of pathogens and mycotoxins creates an environment highly susceptible to infections, inflammation, and reduced nutrient absorption, severely impacting animal

health and performance. In summary, factors such as diet composition, environmental conditions, pathogens, and toxins (endotoxins, mycotoxins) all converge to elevate oxidative stress within the gastrointestinal tract. This multifaceted stress environment disrupts gut integrity, immune function, and nutrient absorption, ultimately impacting overall health and productivity in livestock systems.

1.5.3 Antioxidants

Antioxidants play a crucial role in maintaining intestinal health through mechanisms including scavenging of reactive oxygen species and defense against oxidative stress-induced injuries (Forman and Zhang, 2021). Indeed, the role of both endogenous (derived from the body's metabolism) and exogenous (received through intake) antioxidants are critical in fighting oxidative stress to preserve the function of the gastrointestinal tract, especially under challenging conditions.

A number of enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px), are responsible for the body's endogenous antioxidant defense systems against the effects of ROS (Yu et al., 2016). As SOD catalyzes the conversion of superoxide radicals into hydrogen peroxide, it confers to the first line of defense (Zheng et al., 2023). There are several isoenzymes of SOD located in different cellular compartments. SOD1 in the cytosolic compartment, and SOD2 in the mitochondrial compartment, catalyze dismutation of cell superoxide radicals (Qin et al., 2024b). This compartmentalization is critical for safeguarding cellular structures, such as mitochondria, which are particularly susceptible to oxidative damage due to their involvement in energy

production (Vázquez-Meza et al., 2023). Catalase reacts with the produced hydrogen peroxide, converting it into water and oxygen (Baker et al., 2023). Catalase is predominantly localized in peroxisomes and plays a crucial role in a secondary line of defense by rapidly removing hydrogen peroxide in order to prevent its accumulation to toxic levels (George, 1947). Importantly, this enzyme is especially relevant in enterocytes, as these cells are in direct contact with diets and microbial metabolites, which can induce ROS (Meijnikman et al., 2024). Catalase prevents oxidative stress from exceeding the gut threshold to preserve cell viability and protect intestinal barrier function. Another key enzyme is GSH-Px, which uses GSH as a cofactor to eliminate peroxides and lipid peroxides that are particularly toxic to cells through redox reactions (Brigelius-Flohé and Maiorino, 2013). GSH-Px plays a key role in suppressing lipid peroxidation, in which ROS damage the polyunsaturated fatty acids of the cell membrane, thus disrupting membrane fluidity and integrity. This is particularly important in the intestine, where the enterocyte membranes play an essential role in the absorption of nutrients and barrier integrity. GSH-Px protects the structure of these membranes and accounts for their selective permeability by detoxicating lipid peroxides (Brigelius-Flohé and Maiorino, 2013).

One of the most important endogenous antioxidants is glutathione, a tripeptide formed of glutamate, cysteine and glycine (Georgiou-Siafis and Tsiftsoglou, 2023). Glutathione is abundant in enterocytes and shows multifunctional properties in maintaining cellular redox homeostasis. As a primary substrate of GSH-Px, glutathione is essential for detoxifying peroxides and preventing oxidative stress. It also participates in radical scavenging and helps detoxify xenobiotics through conjugation reactions (Georgiou-Siafis and Tsiftsoglou,

2023). These antioxidant and detoxifying functions are particularly crucial in the gastrointestinal tract, which is frequently exposed to dietary toxins, pathogens, and environmental pollutants. Thus, preserving glutathione levels is crucial for antioxidant defense and general detoxification in the intestinal epithelium. Glutathione synthesis is limited by the availability of cysteine, an amino acid that serves as a precursor in glutathione synthesis. Cysteine or cysteine precursor (methionine) rich diets are demonstrated to increase GSH level and improve gut's tolerance to oxidative stress (Wang et al., 2024a). During phases of increased oxidative stress, such as weaning or disease exposure, demand for GSH can exceed its rate of natural synthesis, highlighting the importance of maintaining GSH homeostasis.

In addition to these core enzymes and non-enzymatic antioxidant GSH, other antioxidant proteins and gut systems also help manage oxidative stress. For example, peroxiredoxins and thioredoxins are key in reducing peroxides and restoring antioxidant capacity (Sadowska-Bartosz and Bartosz, 2023). In particular, peroxiredoxins can degrade hydrogen peroxide and organic hydroperoxides, enhancing intestinal antioxidant defense (Dagdeviren et al., 2022). They partially synergize and support SOD, catalase, and GSH-Px in the neutralization of the different types of ROS.

Exogenous antioxidants obtained from the diet can enhance endogenous defense capabilities and further resist oxidative stress. Dietary antioxidants, including vitamins C and E, selenium, and polyphenols, may also improve the intestinal antioxidant properties. Vitamin E is a fat-soluble antioxidant that protects cellular membranes by preventing lipid

peroxidation and stabilizing free radicals. Vitamin C, a water-soluble antioxidant, further supports the overall antioxidant capacity by supporting vitamin E regeneration and scavenging reactive species (Traber, 2024). Selenium being an important part of GSH-Px enhances its ROS-scavenging activity in intestine (Chen et al., 2023b). Polyphenols, particularly those derived from plants such as flavonoids, promote gut health through the neutralizing of ROS and exert anti-inflammation effects (Kanner, 2023). These compounds have been proven to affect the production of cytokines, which result in reduced inflammation induced by oxidative stress (Winiarska-Mieczan et al., 2023). In addition, polyphenols also contribute to the development of beneficial gut microbiota, which further promotes the stability of the intestinal environment, decreases oxidative pressure and maintains the intestinal barrier function (Xie et al., 2023). They serve in a supportive role, as a complement to the essential role played by endogenous antioxidants to preserve intestinal homeostasis and protect against damage caused by ROS.

1.5.4 Mechanisms of oxidative stress

It is well-established that oxidative stress has significant impact on intestinal health through several cellular mechanisms, which in turn affect the structural integrity of the intestine, nutrient absorption, immune response and overall homeostasis. Nevertheless, the mechanisms are quite complex and involve several interrelated cellular processes.

Lipid peroxidation is one of the first and most serious effects of oxidative stress in the intestine in which ROS react with the polyunsaturated fatty acids of cell membrane. This reaction causes a self-propagating lipid peroxidation chain, generating reactive aldehydes

such as MDA and 4-hydroxynonenal (Zhang et al., 2023). These by-products cross-link membrane proteins, alter membrane fluidity and disrupt cell integrity. In intestinal epithelial cells, lipid peroxidation impairs tight junction proteins (occludin, claudins and ZO-1) (Zeng et al., 2024). The destruction of tight junctions weakens the intestinal barrier, triggering systemic inflammation. In addition, lipid peroxidation by-products act as signaling molecules, exacerbating oxidative stress, activating inflammatory pathways and further amplifying ROS production, thus creating a vicious cycle of oxidative and inflammatory damage (Ngendahayo Mukiza and Dubreuil, 2013).

The nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is a key regulator of the antioxidant response in cells. Under normal conditions, Nrf2 binds to its inhibitor protein, Kelch-like ECH-associated protein 1, in the cytoplasm. During oxidative stress, ROS induce the dissociation of Nrf2 from Kelch-like ECH-associated protein 1, allowing Nrf2 to translocate to the nucleus and where it initiates transcription of antioxidant genes such as glutathione-S-transferase, NAD(P)H dehydrogenase, and heme oxygenase-1 (HO-1) (Suzuki et al., 2023). While the activation of the Nrf2 pathway provides a protective response, excessive oxidative stress can impair its function, reducing the expression of crucial detoxifying enzymes. This impairment weakens cellular resilience against ROS, leaving the epithelial cells vulnerable to oxidative damage and reducing their ability to repair the intestinal barrier. Although compounds like sulforaphane and curcumin have shown promise in activating Nrf2, chronic oxidative stress can diminish the pathway's effectiveness, allowing oxidative damage to accumulate over time (Geertsema et al., 2023).

Oxidative stress serves as a potent trigger for the activation of inflammatory pathways, notably the nuclear factor kappa B (NF-κB) pathway (Shi et al., 2023). ROS act as secondary messengers in this pathway by activating IκB kinase, which phosphorylates the NF-κB inhibitor IκB. Phosphorylated IκB is subsequently degraded, allowing NF-κB to translocate into the nucleus, where it promotes the transcription of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β (Li et al., 2024; Mukherjee et al., 2024). These cytokines amplify the inflammatory response by recruiting immune cells, including macrophages and neutrophils, to the site of tissue damage. Infiltrating immune cells further release ROS and pro-inflammatory mediators, establishing a feed-forward loop that exacerbates oxidative stress. This persistent oxidative-inflammatory milieu disrupts intestinal homeostasis by impairing nutrient absorption, compromising epithelial barrier integrity, and altering gut microbiota composition (Sun et al., 2024). Prolonged activation of NF-κB under conditions of oxidative stress leads to inflammation and the production of ROS, aggravating gut dysfunction and heightening susceptibility to infections and metabolic disturbances (Li et al., 2023a).

The body's redox balance is heavily dependent on GSH, a major antioxidant that scavenges ROS and maintains redox homeostasis within cells. In response to oxidative stress, GSH is rapidly oxidized to its disulfide form (GSSG) (Chai and Mieyal, 2023). The GSH/GSSG ratio serves as a sensitive marker of cellular redox status, with a low ratio indicating high oxidative stress. Prolonged ROS exposure depletes GSH, impairing the cell ability to detoxify ROS effectively (Georgiou-Siafis and Tsiftsoglou, 2023; Giustarini et al., 2023). This depletion is particularly harmful to intestinal cells, as the intestine needs the

synergistic action of glutathione and enzymes such as glutathione peroxidase to prevent oxidative damage to cell membranes. As glutathione levels decline, the detoxification of ROS is impeded, leading to ROS accumulation and cell damage. A reduction in glutathione also means less support for key antioxidant enzymes, which further weakens the antioxidant defenses of the cells, impairs the integrity of the intestinal barrier and exacerbates inflammation (Halliwell, 2024).

Mitochondria are essential for adenosine triphosphate production and are also major contributors of cellular ROS (Martini and Passos, 2023).Under normal conditions, ROS are byproducts of adenosine triphosphate production in the mitochondrial electron transport chain (Nolfi-Donegan et al., 2020). ROS are mainly produced at complexes I and III, where some electrons can leak from the electron transport chain and react with oxygen, forming superoxide radicals (Okoye et al., 2023). These ROS act as signaling molecules in vital processes like gene expression and apoptosis; however, excessive amounts can lead to cellular damage (de Almeida et al., 2022). To control ROS levels, cells use antioxidant defenses such as superoxide dismutase, catalase, and glutathione peroxidase (de Haan et al., 2003). This balance between ROS production and removal helps maintain healthy ROS levels, supporting normal cell function and stability. However, under oxidative stress, the electron transport chain malfunctions, leading to increased electron leakage and enhanced ROS production (Afzal et al., 2023). ROS-mediated mitochondrial DNA and protein damage can disrupt mitochondrial function, creating a vicious cycle of impaired adenosine triphosphate synthesis and ROS production (Shimura, 2023). Mitochondrial-induced apoptosis damages the intestinal epithelial layer, increasing its permeability and exposing

the host to pathogens and toxins (Alula et al., 2023). At the same time, oxidative stress can affect the function of the endoplasmic reticulum, which is essential for protein folding and lipid processing. An oxidative imbalance in the endoplasmic reticulum can lead to the accumulation of misfolded proteins and trigger the unfolded protein response (Ong and Logue, 2023). Although initially aimed at restoring endoplasmic reticulum homeostasis by reducing the protein load and increasing the activity of chaperone proteins, sustained endoplasmic reticulum stress can activate apoptotic pathways and further generate ROS, thereby exacerbating cell damage (Chen et al., 2023a). Sustained endoplasmic reticulum stress can also stimulate inflammatory pathways, especially NF-κB and mitogen-activated protein kinase, which amplify ROS production and perpetuate the cycle of oxidative damage (Akhter et al., 2023). Mitochondrial and endoplasmic reticulum dysfunction can cause intestinal cell apoptosis and adenosine triphosphate depletion, which can severely damage intestinal integrity and increase inflammation (Li et al., 2023c).

1.6 Inflammation and gut health

Gastrointestinal inflammation is a complex, multifaceted process that can affect intestinal function, nutrient absorption, immune balance and overall health. This section will explore the interplay between oxidative stress and inflammation in the gut, the factors that contribute to gut inflammation and the mechanisms by which inflammation is expressed in the gastrointestinal system. Gut inflammation involves immune cell activation, cytokine release and molecular signaling pathways. When these processes are dysregulated, impairment of intestinal integrity and function occurs.

1.6.1 Association between oxidative stress and inflammation

Oxidative stress and inflammation are closely connected biological processes. In the gastrointestinal tract, excess ROS induce cellular damage that triggers inflammatory signaling pathways (Campbell and Colgan, 2019). ROS compromise key cellular components and directly impairing cell function and structural integrity, which increase intestinal permeability (Aviello and Knaus, 2017). Compromised barrier allows antigens and endotoxins from the lumen to enter the systemic circulation, further amplifying inflammatory responses and recruiting immune cells to the site of damage (Ma and Morel, 2022). When ROS levels overwhelm the gut's antioxidant defense, a cycle of oxidative stress and inflammation is established, perpetuating tissue damage and barrier dysfunction. In addition to cellular damage, ROS play a signaling role in activating key transcription factors that are involved in inflammation (Priya Dharshini et al., 2020). NF-κB is activated in response to oxidative stress and promotes the transcription of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β (Li et al., 2020a). This amplification loop between oxidative stress and inflammation forms a self-perpetuating cycle that exacerbates gut health issues, contributes to chronic inflammation, and leads to structural and functional deterioration of the gut barrier (Mazumder et al., 2022).

1.6.2 Common factors causing inflammation in the gut

Multiple internal and external factors contribute to inflammation in the gut, particularly in intensive farming environments. Dietary composition plays a significant role, as diets high in saturated and trans fats with low fiber content can promote inflammatory responses (Ye et al., 2021). High-fat diets generate pro-inflammatory byproducts, whereas low fiber

intake limits the production of short-chain fatty acids that are the key compounds in supporting gut barrier integrity and mitigating inflammation (Bilal et al., 2022). Without sufficient short-chain fatty acids, the gut loses the vital anti-inflammatory support, making it more prone to inflammatory responses (Zhang et al., 2022c). Environmental stressors such as high-density living conditions, fluctuations in temperature, and inadequate sanitation further exacerbate intestinal inflammation (Irwin, 2023). Another important factor driving inflammation is dysbiosis of gut microbiota. The balance of gut microbiota is directly involved in immune stability, but dysbiosis leads to overgrowth of pathogenic bacteria, which produce endotoxins that activate inflammatory signaling (Baldelli et al., 2021). This kind of imbalance causes a disturbance in the production of cytokines, leading to enhanced inflammatory stress and reduced barrier function of the gut (Barbara et al., 2021). Pathogen (*E. coli* and *Salmonella*) exposure also induces gut inflammation (Zhao et al., 2022). These pathogens destroy epithelial cells, producing toxins that stimulate immune responses through the release of pro-inflammatory cytokines (Huang et al., 2022). This immune activation disrupts tight junctions between epithelial cells, increasing gut permeability (Stephens and von der Weid, 2020). These factors work together to impair the integrity of the intestinal structure and function, weaken its innate defenses and drive it into chronic inflammation.

1.6.3 Mechanisms of inflammation

The inflammatory response in the intestines is a complex interplay between innate and adaptive immune pathways (Deets and Vance, 2021). Cytokines, immune cells and signaling pathways that respond to oxidative damage and microbial invasion play an

integral role in coordinating the body's defense mechanisms, but they can also exacerbate tissue damage in chronic diseases. The inflammatory response is mediated by key cytokines such as TNF-α, IL-6, and IL-1β. These cytokines are released by immune cells, specifically macrophages and neutrophils, in response to increasing levels of ROS and microbial invasion (Pidwill et al., 2021). For example, TNF-α promotes this inflammatory feedback loop by inducing continued ROS generation in tissues, thus further promoting tissue damage (Velatooru et al., 2021). While IL-6 and IL-1β also promote inflammation by attracting more immune cells to the site of injury, they increase the secretion of cytokines and lead to increased levels of ROS (Pyrillou et al., 2020). This cytokine cascade promotes chronic inflammatory status that impairs gut health and integrity.

NF-κB is a critical transcription factor in inflammatory gene expression induced by oxidative stress and pathogen invasion. In this pathway, ROS serve as secondary messengers that promote the activation of IκB kinase and consequently results in the phosphorylation and degradation of IκB, the inhibitor of NF-κB (Wang et al., 2016). In this way, following being released from its inhibitor, NF-κB migrates into the nucleus and activates the production of proinflammatory cytokines. In chronic inflammatory conditions, the sustained activation of NF-κB promotes low-grade inflammation that gradually weakens the gut barrier, increasing susceptibility to pathogens and toxins.

Toll-like receptors and other pattern recognition receptors are crucial in detecting pathogen-associated molecular patterns on invading microbes (Janssens and Beyaert, 2003). The activation of Toll-like receptors initiates downstream inflammatory processes,

including cytokine release and ROS production, as a rapid response to infection. While Toll-like receptor signaling is vital for pathogen defense, unchecked Toll-like receptor activation can lead to chronic inflammation and tissue damage. For instance, Toll-like receptor 4 is particularly responsive to lipopolysaccharides from Gram-negative bacteria, triggering significant cytokine release and ROS production that can contribute to prolonged inflammation (Cochet and Peri, 2017).

The inflammasome, especially the NLR family pyrin domain containing 3 inflammasome, is a multi-protein complex that responds to cellular stress signals, including ROS and microbial toxins (Jo et al., 2016). Once activated, the inflammasome facilitates the processing of the pro-inflammatory cytokines IL-1β and interleukin-18 into their active forms, amplifying the inflammatory response. This activation can exacerbate intestinal inflammation and has been implicated in the pathogenesis of inflammatory bowel diseases, where persistent inflammasome activation contributes to chronic mucosal damage, impaired epithelial barrier function, and sustained immune dysregulation (Zhen and Zhang, 2019).

1.7 Management of gut health in swine

The maintenance of gut health is the most important aspect in the swine industry because it is directly related to animal welfare, productivity, and disease resistance. The gastrointestinal tract is an organ that is highly important for absorption, immune function, and growth. It is quite easily exposed to infections and environmental stress. Maintaining

optimal gut health is challenging in modern swine production due to high stocking density and increased risk of pathogen exposure under poor hygiene conditions.

1.7.1 Challenges in the swine industry

Modern swine production faces many issues that challenge gut health, among which high-density housing is the most important. This approach is designed to optimize space for economic profits but at the same time raises the stress level for animals and leads to the rapid spread of gastrointestinal pathogens (Zeng et al., 2022). High-density farming weakens the immune system by raising stress hormones like cortisol, which suppress immune responses and make pigs more susceptible to gut infections caused by pathogens such as *E. coli*, *Salmonella*, and other enteric bacteria (Li et al., 2022b). Another major challenge that swine will encounter early is weaning. The weaning process may introduce physiological and psychological stress in animals, leading to poor feed intake, imbalance of gut microbiota, and disruption of gut permeability (Tang et al., 2022). Such a phase leads to a very common disease of the piglet called post-weaning diarrhea due to enteric pathogens like *E. coli*. Weaning stress also imposes a challenge to the immature gut immune system, inducing inflammation, oxidative stress, and nutrient malabsorption, affecting growth rate and health outcome (Qiao et al., 2023).

The composition of the diet further influences gut health in swine. High-energy diets, which are usually designed to maximize growth, often have inadequate levels of fiber, leading to reduced production of beneficial SCFAs that support gut barrier integrity and microbiota balance (Deehan et al., 2024). As a result, these low-fiber diets can disturb gut

microbiota, impair immune function, and make animals vulnerable to dysbiosis, therefore increasing inflammation and oxidative stress (Gill et al., 2021). Additionally, seasonal and temperature variations in agricultural regions, particularly during summer, introduce stressors that not only affect digestive function and feed utilization but also create additional gut health challenges (Lee et al., 2020). Mycotoxins in feed are another frequent environmental contaminant. Mycotoxins, including aflatoxin and fumonisin, threaten gut integrity, as they promote oxidative stress and immune responses in pigs resulting in a higher susceptibility to gastrointestinal infections and systemic diseases (Mavrommatis et al., 2023). Indeed, better control of these environmental and dietary challenges is critical to supporting gut health, reducing disease incidence, and optimizing growth and productivity.

1.7.2 Use of antibiotics in the past

Antibiotics are very important to gut health and infectious diseases management in the swine industry. Low-dose antibiotic growth promoters have been used in practice for several decades to improve body weight gain and feed conversion efficiency, while reducing incidence of disease (Brown et al., 2017). Antibiotics are efficient in keeping gut pathogens low and reducing subclinical infections. Generally, this improved gut health and growth performance of the animal (Luise et al., 2022). Preventive use of antibiotics also contributed to reduced mortality, especially during sensitive periods such as at weaning, when piglets are more susceptible to gastrointestinal disturbance (Diana et al., 2019). Antibiotics improve gut health through multiple mechanisms: 1) antibiotics decrease the loads of pathogenic bacteria in the gastrointestinal tract which limit the competition for

nutrients; 2) antibiotics prevent the pathogenic bacteria overgrowth, which help to maintain a healthy microbial balance, reduce inflammation, and facilitate better absorption of nutrients; 3) preventing bacterial overload reduced the metabolic cost of responding to infection, allowing energy to be diverted towards growth rather than disease defense. In contrast, therapeutic use of antibiotics involves higher doses used for a defined period to treat bacterial infections. This is essential for managing acute diseases such as respiratory or enteric infections and is generally administered based on clinical diagnosis. Unlike growth promotion, therapeutic antibiotic use is typically time-limited and targeted, though concerns remain regarding antimicrobial resistance even with judicious application.

1.8 Exploring alternatives to antibiotics

Because of the increased prevalence of antibiotic-resistant bacteria, the animal production industry has been urged to transition from using antibiotic growth promoters to alternative solutions. In particular, the swine industry has struggled to keep their animals productive and healthy without the use of antibiotics. Antibiotic alternatives are crucial for sustainable swine production, animal welfare, and satisfying consumers who prefer meat from animals raised without in-feed antibiotics.

1.8.1 The need to explore alternatives to antibiotics

Historically, antibiotics have played a dual role in swine production. They have not only treated and prevented infections but have also been used as growth promoters to improve feed efficiency and growth rates. However, the widespread use of antibiotics in livestock production raised significant public health concerns, primarily due to the emergence of

antibiotic-resistant bacteria (Muteeb et al., 2023). The overuse and misuse of antibiotics in animal agriculture led to the development of multidrug-resistant pathogens, which posed a risk not only to animal health but also to human health through zoonotic transmission (Chechet et al., 2023). Antibiotic residues in meat products and the spread of resistant bacteria to humans prompted regulatory agencies to take actions (Monger et al., 2021). The European Union banned the use of antibiotic growth promoters in 2006, and similar restrictions were followed in other parts of the world, including the United States and Canada (Ager et al., 2023). Phasing out of antibiotic growth promoters proves challenging for the pig industry, particularly gut health and growth performance without antibiotic usage. This highlights the need to identify alternative approaches to promote gut health, prevent disease, and sustain productivity.

1.8.2 Current approaches to manage gut health

Gastrointestinal health and performance are challenged in the absence of antibiotic growth promoters and need a multi-faceted approach. This encompasses management and nutritional strategies that limit stress and promote a healthy intestinal environment. Optimal stocking densities, good ventilation, and keeping animals in comfortable living conditions form the cornerstone of good husbandry practices that minimize stress-related intestinal problems. It has been shown that a reduction in stress levels increases immune function and reduces the necessary application of antimicrobial treatments (Kim et al., 2022b). Some studies have found that better integrity of gut associated with better environmental conditions could improve pigs resistance to infectious disease without the use of antibiotics (Gardiner et al., 2020). From a nutritional perspective, precision feeding

is part of restoring immunity and gut functions (Sasson et al., 2021). It controls the diet to just an appropriate amount of energy, proteins, and micro-nutrients, decreasing metabolic stress to ensure enhancement in immune functions (Shao et al., 2021). Feed quality control is another major point, particularly reducing mycotoxin contamination, which may induce oxidative stress response and alter gut integrity. Nutritional strategies including the addition of high-quality proteins and bioactive compounds are also useful in maintaining gut health, especially during weaning (Ma et al., 2022). Additionally, the use of natural compounds and feed additives as substitutes for antibiotics is gradually becoming a promising strategy for gut health.

1.8.3 Common antibiotic alternatives

Various feed additives have recently proven their efficacy as antibiotic alternatives, each showing a unique mechanism to promote gut health, immunity, and growth performance in swine production. Diets without antibiotics have led to the use of alternatives, which consist of probiotics, prebiotics, organic acids, phytogenics and enzymes (Silva Júnior et al., 2020). Probiotics are beneficial bacteria that can colonize the gut and inhibit the overgrowth of pathogenic microbes, supporting a balanced gut microbiota (Shi et al., 2021). In weaned piglets, *Lactobacillus plantarum* CAM6 administertion improved growth performance and immune parameters of the animals by increasing the level of IgA in serum, without negative effects on animal health (Betancur et al., 2020). This suggests that probiotics may be useful alternatives of antibiotic growth promoters to modulate gut homeostasis and boost immune ability during the stress of weaning. Prebiotics are non-digestible fibers that selectively nurture pro-health bacteria of the gut (Hayhoe et al., 2022).

They increase the production of short-chain fatty acids such as butyrate, which are important energy substrates for colonocytes as well as critical mediators of gut barrier integrity and anti-inflammatory effects. By stimulating a beneficial microbiota profile, stabilizing microbiota and preventing the colonization by pathogenic bacteria, prebiotics enhance gut health (Li, 2021). Other potential antibiotic alternatives are organic acids, including butyrate and formic acid (López-Colom et al., 2020; Dahmer et al., 2022). They can lower pH in the gastrointestinal tract, hence restricting the growth of pathogenic bacteria. Some studies have shown that formic acid significantly reduce diarrhea score and improve feed conversion ratio in weaned piglets, suggesting its ability to act as a strong alternative to antibiotics for gut health intervention (Dahmer et al., 2022). Phytogenics are plant-derived compounds such as essential oils and flavonoids that can provide many benefits in diets for pigs (Chang et al., 2022). They have antimicrobial, anti-inflammatory and antioxidant properties, modulate the gut microbiota, and help to improve nutrient absorption and feed intake (Chang et al., 2023). Some studies have indicated that oregano essential oil may serve as a bioactive compound with antimicrobial properties and may be a natural alternative to antibiotics to suppress Gram-positive and Gram-negative pathogens without compromising growth performance (Serrano-Jara et al., 2024). To enhance the digestibility of nutrients, enzymes like β-mannanase are employed for the degradation of complex constituents of the feed that may favor the proliferation of harmful bacteria (Jang et al., 2024b). Supplementation with β-mannanase has been shown to greatly reduce post-weaning diarrhea in piglets, hence minimizing the use of antibiotics and supporting optimal gut health in this critical phase (Jang et al., 2024a). Overall, the absence of antibiotic growth promoters requires an integrated approach to support gut health and productivity in

swine production. Improved management practices, precision nutrition, and viable alternatives including probiotics, prebiotics, organic acids, phytogenics, and enzymes will enable the swine industry to reduce antibiotic use in a sustainable manner that continues to ensure good animal welfare and performance.

1.8.4 Probiotics

Probiotics are one of the most widely researched and used compounds in animal feed, with antibiotic-like effects. Probiotics are live microorganisms, most commonly beneficial bacteria, which provide health benefits to the host when supplied in sufficient amounts (Muwonge et al., 2021). Most strains of probiotic bacteria administered within swine are from *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and *Enterococcus* (Castillo Zuniga et al., 2024). Probiotics contribute to gut health through various mechanisms. Probiotics play a role in maintaining a balanced gut microbiota by competing with pathogenic bacteria for nutrients and adhesion sites in the gut lining (Muwonge et al., 2021). This competitive exclusion is important as it prevents the colonization of pathogenic bacteria such as *E. coli* and *Salmonella*, which are well-known to cause gastrointestinal disorders (Song et al., 2023). A recent study has shown that *Lactobacillus plantarum* can produce antimicrobial compounds such as bacteriocins which competitively exclude the growth of these pathogenic microorganisms, reducing gut infection and helping maintain the balance of gut microbiota (Chen et al., 2022a). Probiotics not only inhibit pathogenic bacteria through competitive exclusion but also actively modulate the host immune system. They influence the mucosal immune response by stimulating the production of secretory IgA, which serves as the first line of defense against enteric pathogens. Among probiotic strains,

Bifidobacterium species have been noted for their ability to enhance sIgA levels, thereby strengthening the gut's immune barrier and promoting intestinal homeostasis (Abdi and Ranjbar, 2022). Probiotics also interact with gut-associated lymphoid tissues, which in turn enhances the action of immune cells including the macrophages and dendritic cells (Shi et al., 2020). In addition, probiotics strengthen intestinal barrier function by enhancing the expression of tight junction proteins (Bhat et al., 2020). Thus, probiotics prevent the translocation of toxins and pathogens across the intestinal barrier, thereby reducing systemic inflammation and improving gut health. A recent study has shown that *Lactobacillus rhamnosus* GG enhances the expression of tight junction proteins, resulting in a stronger gut barrier and preventing leaky gut (Mao et al., 2020). Moreover, probiotics produce beneficial metabolites like short-chain fatty acids, such as butyrate, propionate, and acetate. These short-chain fatty acids are the energy source of colonocytes, maintain the gut-barrier functions, and have anti-inflammatory effects (Kim, 2021). For example, butyrate has been studied in its important role in promoting epithelial cell proliferation and reducing inflammatory responses in the gut, contributing to gut health (Liang et al., 2022). Overall, probiotics act through a variety of mechanisms, including direct antimicrobial effects, alteration in the immune response, as well as improvement of gut barrier functions. It is worth noting that such an addition in swine production not only results in the reduction of antibiotics use, but also improves growth performance, feed utilization and animal health.

1.8.5 Fibers

Dietary fibers, as functional constituents of swine diets, have gained increasing interest as promising non-antibiotic strategies to enhance gut health and promote growth performance.

There are certain types of fibers that have been identified in recent research that are key to maintaining and improving gut health. Dietary fibers are generally divided into soluble and insoluble types. Those with proven health benefits are referred to functional fibers. Soluble fibers, including inulin and pectin, dissolve in water and are fermented by gut microbiota to produce short-chain fatty acids (Wang et al., 2019a). Insoluble fibers, which include cellulose and hemicellulose, do not dissolve in water but add bulk to the stool, promoting regular bowel movements and preventing constipation (Gill et al., 2021). Functional fibers (resistant starches and oligosaccharides) have effects beyond those of traditional dietary fibers, meaning that functional fibers have specific beneficial effects in the digestive tract, often in connection with gut microbiota, and therefore can be used to target health in a microbiota-dependent manner (Wan et al., 2021). The beneficial effects of dietary fibers are mainly due to several mechanisms leading towards gut environmental improvement. It is known that gut bacteria can ferment soluble fiber and produce short-chain fatty acids. Beyond the production of short-chain fatty acids, fibers also stimulate the growth of beneficial gut bacteria and prevent the growth of detrimental bacteria (Rezende et al., 2021). This selective stimulation of beneficial bacteria is called a prebiotic effect, and it contributes to a balanced gut microbiota. Certain animal studies reveal that inulin (a kind of soluble fiber contained in certain foods like chicory root and onions) can increase *Bifidobacterium* abundance in the gut (Wang et al., 2020a). *Bifidobacterium* is known for its role in enhancing immune function and maintaining gut homeostasis (Sun et al., 2020b). Such beneficial bacteria also competitively exclude pathogenic bacteria, minimizing gastrointestinal infections and further enhancing gut health (Manzoor et al., 2022). Another important mechanism by which dietary fibers promote gut health is by improving gut

motility and reducing the risk of digestive disturbances (Gill et al., 2021). By improving gut motility, insoluble fiber reduces the retention time of ingested feed within the gut. This limits the proliferation of pathogenic bacteria that generally thrive in environments that are not dynamic. The shortened retention time also works to mitigate the accumulation of damaging metabolites like ammonia and bile acids that can continuously damage the gut lining and promote inflammation. Functional fibers such as resistant starch and β-glucans have also been recognized to be of significance in improving gut health. Resistant starch is the starch that resists digestion within the small intestine and gets fermented in the colon, producing SCFAs. Previous studies have identified that resistant starch can lower gut pH, which is less favorable to pathogenic bacteria (Ta et al., 2021). In addition, β-glucans from cereals (oats and barley) have immunomodulatory effects and are helpful for gut immunity. They have been found to enhance the activity of various immune cells, including macrophages and neutrophils, improving the intestinal resistance to infection (Stothers et al., 2021; Braian et al., 2023).

Dietary fiber supplementation has been shown to improve both growth performance and health in pigs. Some studies have shown that a high-fiber diet is associated with a reduction in diarrhea score in pigs (Ma et al., 2024). This reduction is largely attributed to the role of fibers in promoting a balanced gut microbiota and enhancing the gut barrier. Besides, dietary fibers can improve gut morphology, including increased villus height and reduced crypt depth (Hedemann et al., 2006). Healthy intestinal morphology provides a larger absorption surface area that is important for the growth and development of pigs. Overall, dietary fibers may be proposed as one of the beneficial antibiotic alternatives that improve

gut functions and enhance growth performance in pigs. Although dietary fibers have been highly recognized for their beneficial effects on gut health and growth performance, less attention has been directed toward the role of fiber in mitigating oxidative stress in swine. oxidative stress impairs gut barrier function, depresses nutrient absorption, heightens susceptibility to infection, thus presenting a major challenge toward swine production. Therefore, more research would be required to uncover the underlying mechanisms through which fibers mediate these effects as well as to identify the optimal type of fibers for alleviating oxidative stress.

CHAPTER TWO. RATIONALE, HYPOTHESIS AND OBJECTIVES

2.1 Rationale

2.1.1 Effects of high-fiber and low-fiber diets on intestinal oxidative stress in pigs

Oxidative stress is a critical factor in intestinal dysfunction, contributing to compromised gut health, increased permeability, and inflammation (Kim et al., 2012; Fukui, 2016). In pigs, oxidative stress can arise from multiple sources, including dietary composition, pathogenic infections, and microbial imbalances (Wu et al., 2021b; Rodrigues et al., 2022; Lian et al., 2024). Understanding the mechanisms by which oxidative stress is regulated in the intestine is essential for developing effective nutritional and therapeutic strategies. Traditionally, dietary fiber was considered to have low nutritional value due to its limited digestible energy (Renaudeau et al., 2013). However, emerging research highlights its functional role in gut health, particularly in modulating gut microbiota and promoting intestinal integrity (Temple, 2000; Adom et al., 2003). Some studies have shown that high-fiber diets can promote the production of short-chain fatty acids, which support beneficial bacteria growth, enhancing the gut's antioxidant capacity and reducing ROS production (Han et al., 2023). However, limited information is available regarding how different fiber levels regulate oxidative stress in the intestines of pigs.

2.1.2 Impact of enterotoxigenic *Escherichia coli* infection on intestinal oxidative stress in pigs

Enterotoxigenic *Escherichia coli* infection is a significant global concern, particularly among post-weaning pigs, leading to severe diarrhea, impaired nutrient absorption, and growth retardation (Foster-Nyarko and Pallen, 2022; Pokharel et al., 2023). Studies have shown that ETEC infection can disrupt intestinal integrity by increasing the permeability

and reducing the expression of tight junction proteins such as ZO-1 and occludin (Duan et al., 2022). Increased oxidative stress and inflammation can exacerbate gut damage after infection. Although it is known that ETEC infections impair the intestinal barrier, the specific role of oxidative stress in intestinal health, particularly in the context of ETEC infections, is not well understood. Oxidative stress, a condition resulting from an imbalance between ROS production and antioxidant defenses, is a critical factor in intestinal dysfunction (Martemucci et al., 2022). Glutathione, a crucial antioxidant in cellular defense, plays an essential role in maintaining redox balance and mitigating ROS-induced cellular damage (Chai and Mieyal, 2023). Its synthesis relies on enzymes such as glutamate-cysteine ligase (GCL) and glutathione synthetase (GS), both of which are regulated by Nrf2 (Lu, 2013). Despite the importance of glutathione in protecting intestinal cells, little information is available regarding how ETEC infection impacts glutathione biosynthesis in the intestine and the molecular mechanisms remain unclear (Tang et al., 2019).

2.1.3 Effect of probiotics on intestinal oxidative stress in pigs

In recent years, probiotics have garnered significant interest in their potential benefits in enhancing gut health and modulating oxidative stress. Previous studies suggest that probiotics can strengthen antioxidant defense by promoting SCFAs production and supporting a balanced gut microbiome, which together enhance intestinal integrity (Zhang et al., 2020). Probiotics have also been shown to improve tight junction protein expression, suppress inflammatory cytokines such as IL-6 and TNF-α (Zheng et al., 2022). Despite these promising findings, limited research is available on how probiotics influence oxidative stress under ETEC infection conditions. Some studies suggest probiotics may

decrease the mRNA expression of Nrf2 in the jejunum and ileum under ETEC infection, while others report a significant increase of Nrf2 (Raheem et al., 2021; Wu et al., 2022).

This research integrates three key aspects of gut health (dietary fiber, ETEC infection, probiotics) to provide a comprehensive understanding of oxidative stress regulation in pigs. By investigating how fiber modulates antioxidant defenses, how ETEC infection disrupts oxidative balance, and how probiotics restore intestinal homeostasis, this research aims to bridge existing knowledge gaps and support the development of targeted nutritional interventions. The findings will have practical implications for improving intestinal health, reducing the reliance on antibiotics, and enhancing overall productivity in swine production systems.

2.2 Hypothesis

We hypothesized that: (1) fiber supplementation can improve intestinal health by reducing intestinal oxidative stress; (2) ETEC infection impaires glutathione biosynthesis and Nrf2 signaling pathway, causing oxidative stress; (3) probiotics reduce oxidative stress through glutathione biosynthesis and Nrf2 signaling pathway and improve gut function after infection.

2.3 Objectives

The overall objective of our research was to investigate the impact of oxidative stress on gut health as well as the underlying mechanisms, and to explore whether dietary fiber and

probiotics could alleviate oxidative stress, thereby improving gut health. The specific objectives were

Study 1: To investigate the effects and the mechanisms of fiber on oxidative stress and inflammatory response in gut health.

Study 2: To investigate the effect of ETEC infection on intestinal oxidative stress and its underlying mechanisms.

Study 3: To investigate the effect of probiotics on intestinal oxidative stress and the underlying mechanisms.

To address these objectives, this thesis comprises three integrated studies that collectively investigate the regulatory mechanisms of oxidative stress in the gut and explore nutritional interventions for intestinal health. Chapter 3 (Manuscript I) examines the effects of high- and low-fiber diets on oxidative stress and growth performance in growing-finishing pigs. Chapter 4 (Manuscript II) explores how ETEC infection induces oxidative stress and impairs antioxidant defenses, particularly focusing on the Nrf2-GSH axis in weaned piglets. Finally, Chapter 5 (Manuscript III) evaluates the protective effects of *Bacillus licheniformis* HG76 in mitigating oxidative stress and inflammation during ETEC challenge. These results are further synthesized in Chapter 6 (General Discussion), which integrates the findings across the three studies to provide broader insights into redox

regulation and nutritional strategies. Finally, Chapter 7 summarizes the major conclusions, outlines study limitations, and proposes future research directions.

CHAPTER THREE: MANUSCRIPT I

Effects of high- and low-fiber diets on intestinal oxidative stress in growing-finishing pigs

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3.1 Abstract

Feed is the most expensive facet of commercial pork production. In order to reduce feed costs, using high-fiber ingredients has become a common practice. Moderate levels of fiber can maintain intestinal physiological function and promote intestinal health. Oxidative stress is linked to impaired nutrient absorption and growth performance. This study investigated the effects of high-fiber and low-fiber diets on growth performance and intestinal oxidative stress parameters in growing-finishing pigs. Forty growing pigs with initial body weight (27.07 ± 1.26 kg) were randomly assigned to 2 treatment groups with 10 replicates of 2 pigs per pen. Pigs were weighed on day 35, 42, and 70. The feed intake was recorded to calculate growth performance parameters. On day 70, eight pigs in each treatment group were randomly selected and euthanized to obtain jejunum to measure oxidative stress status. Pigs fed a high-fiber diet were heavier than those fed a low-fiber diet on day 70 ($P < 0.05$). During the whole feeding period, pigs fed a high-fiber diet had a higher average daily gain than those fed a low-fiber diet ($P < 0.05$). The low-fiber diet resulted in increased levels of malondialdehyde ($P < 0.05$) in the jejunum, suggesting that the low-fiber diet contributed to oxidative stress in the jejunum. The low-fiber diet also led to a significant increase in glutathione and oxidized glutathione levels ($P < 0.05$) in the jejunum, indicating that pigs fed a low-fiber diet needed to produce more antioxidant substances to cope with oxidative stress in the intestine. This was accompanied by a significant increase in the expression of glutathione synthesizing enzymes in the jejunum of the low-fiber group ($P < 0.05$). These results suggest that the high-fiber diet can improve growth performance and maintain intestinal health in growing-finishing pigs by reducing intestinal oxidative stress.

Key words: fiber, growing-finishing pigs, growth performance, intestine, oxidative stress

3.2 Introduction

Pork is an essential source of animal protein in human nutrition (Pereira and Vicente, 2013). As the population increases and living standards rise, the demand for better-quality pork increases. Although the animal's genetic makeup determines its growth state, animal feed plays a vital role in animal production (Rauw et al., 2020). Animal feed counts for 60% to 70% of total animal production costs (Renaudeau et al., 2013). In order to reduce feed costs, the use of high-fiber ingredients and co-products has become a common practice (Fontenot et al., 1983). Traditionally, corn and soybean meals have been the main components of pig feeds in large parts of the world, providing most of the energy and nutrients required for pigs. Other grains such as barley, wheat, oats, and byproducts including bran, hulls, and distiller's grains are high in energy and nutrients but are fibrous in nature (McKEE and Latner, 2000). When these byproducts are added to swine diets, the diets inevitably shift from high starch diets to diets containing less starch and more non-starch polysaccharides (Hsu et al., 1987; Jaworski and Stein, 2017). Generally, in traditional nutritional studies, fiber-rich feeds are often considered to have lower nutritional value because of their lower digestible energy or amino acid levels than corn or soybean meals (Jørgensen et al., 1996; Jha and Berrocoso, 2015; Jarrett and Ashworth, 2018). As a result, the proportion of high-fiber ingredients in commercial pig diets is low, which leads to underutilization and waste of fiber-rich ingredients. Recent animal nutrition studies have identified the positive effects of fiber in animal production. For example, intestinal microorganisms can ferment fiber to produce volatile fatty acids, providing pigs energy (Zhao et al., 2020). In addition, pregnant sows require a certain amount of fiber in their diets to prevent constipation and prolong satiety (Wenk, 2001). Most importantly, diets for pigs must contain a certain amount of

fiber to maintain the physiological functions of the digestive system (Wu et al., 2018). In addition, fiber has been shown to contain phytochemicals that may act as antioxidants (Temple, 2000; Adom et al., 2003). Therefore, it remains to be investigated whether fiber can act as an antioxidant to protect the intestinal tract of pigs from oxidative stress and maintain intestinal health and function.

The intestine is highly vulnerable to oxidative stress injury because the intestine cells contain many mitochondria that are the major sites for ROS production (Figueira et al., 2012; Liu et al., 2017). Free radicals can disrupt the intestinal structure and disturb the microbial balance, resulting in decreased feed intake and delayed or reduced weight gain, thus significantly reducing the economic efficiency of the pig industry (Liu et al., 2020b; Han et al., 2021; Qiu et al., 2021). Oxidative damage occurs when the antioxidant system is unable to eliminate excess ROS from the host body (Li et al., 2020c). Therefore, maintaining redox balance is crucial for intestinal health. In pig production, numerous substances and environmental factors can cause the body to produce large amounts of free radicals, leading to oxidative damage in animals (Puppel et al., 2015). Proteins are the most frequent targets of ROS attacks, as they are essential components of tissues and are involved in many physiological processes in the host (Anjum et al., 2015). Moreover, lipid peroxidation can damage normal cellular functions by affecting the structure and fluidity of biological membranes and the permeability of cell walls (Catalá and Díaz, 2016). These, in turn, affect intestinal health and animal growth. Currently, little information is available regarding the regulation of intestinal oxidative stress by fiber. Therefore, this study investigated the effects of fiber on growth performance and intestinal oxidative stress

parameters in growing-finishing pigs. We hypothesized that fiber might regulate growth performance and oxidative stress in growing-finishing pigs.

3.3 Materials and methods

The University of Manitoba's Animal Care Committee reviewed and approved the experimental and animal care protocols (AC#F21-002). In addition, all pigs were cared for following the Canadian Council on Animal Care guidelines (CCAC, 2009).

3.3.1 Animals, treatments, and experimental design

A total of 40 growing pigs (56 d old TN Tempo [Large White] × TN70 [Large White × Landrace]; 20 barrows and 20 gilts with initial body weight [BW] of 27.07 ± 1.26 kg) were fed either low-fiber (corn-soybean meal-based diet) or high-fiber (wheat-canola meal-based diet) diets. Pigs were housed in a temperature-controlled room and randomly assigned to two treatments with a total of 10 pens per treatment and two replicates per pen. Fans and heaters maintained the room temperature at 22.0 ± 2.2 °C throughout the experiment. Pigs were fed experimental diets in three phases (25 to 50, 50 to 75, and 75 to 110 kg), with ad libitum feeding using stainless steel feeders and ad libitum drinking water provided using nipple drinkers. Phase duration was predicted using Topigs Norsvin (Topigs Norsvin, Oak Bluff, MB, Canada) growth performance data on Tempo pigs. The current study was conducted at the TK. Cheung Centre at the University of Manitoba.

3.3.2 Diet and feed ingredients

In the diets of growing-finishing pigs, fiber content increased with the addition of oat hulls and soybean hulls. In addition, vegetable oil was added to high-fiber diets to keep the net energy (NE) content consistent. The high- and low-fiber diets were formulated to meet or exceed the NRC (2012) recommendations for growing-finishing pigs (Table 3. 1). High- and low-fiber diets were mixed at the Glenlea Mill (Glenlea Swine Research Unit, University of Manitoba) prior to each experimental phase. And at this time, samples of experimental diets were collected and stored in a -20 °C refrigerator before wet lab analysis. High- and low-fiber diets were submitted to the Central Testing Laboratory (Central Testing Laboratory Ltd, Winnipeg, MB, Canada) for dry matter (method 930.15; AOAC International, 2000), crude protein (method 990.03; AOAC International, 2000), crude fat (method Am 5-04; AOCS, 2009a), crude fiber (method Ba 6a-05; AOCS, 2009b), acid detergent fiber (ADF; Ankom method, 2006a), neutral detergent fiber (NDF; Ankom method, 2006b), and acid insoluble ash (method 942.05; AOAC International, 2000) were determined. In addition, starch was analyzed by Megazyme total starch determination kit (K-TSTA; method 996.11; AOAC International, 2000). Gross energy were measured by bomb calorimetry (Model 6400; Parr Instruments, Moline, IL). Digestible energy (DE) and metabolizable energy (ME) were estimated based on gross energy, assuming that DE equals 88% of GE and ME equals 96% of DE (NRC, 2012). Non-starch polysaccharides were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst et al. (1994) and Ndou et al. (2015). Non-fiber carbohydrates were calculated as 100 - (crude protein+NDF+crude fat+ash) (Hall, 2000). Finally, the chemical composition of high- and low-fiber diets was

shown in Table 3.2, and the non-starch polysaccharide (NSP) composition of soya hulls and oat hulls was shown in Table 3.3.

Table 3.1 Composition of experimental diets, as-fed basis, g·kg^{-1}

Item	(25-55 kg)		(55-75 kg)		(75-110 kg)	
	Low fiber	High fiber	Low fiber	High fiber	Low fiber	High fiber
Ingredients, kg						
Corn	449.86	332.76	434.07	354.4	383.85	409.83
Wheat	194	185	208	162	261	97
Soybean meal	194	200	103	111	55	77
Canola meal	50	50	75	75	75	75
Vegetable oil	7.5	38.5	2.5	32		26
Barley	75	75	150	150	200	200
Soya hulls		55		60		61
Oat hulls		35		30		30
Limestone	9	8	9	7	9	7
Dicalcium phosphate	5	5	4	4	3	4
Salt	5.3	5.3	4.75	4.75	4.45	4.5
Copper sulfate	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin premix[1]	1.0	1.0	1.0	1.0	0.8	0.8
Mineral premix[2]	1.0	1.0	1.0	1.0	1.0	1.0
Choline Chloride 70	0.3	0.3	0.3	0.3		
L-Lysine-HCl	4.08	3.88	4.19	3.96	4.25	3.75
DL-Methionine	1.32	1.50	0.76	1.00	0.38	0.72
L-Threonine	1.50	1.50	1.50	1.50	1.47	1.39

L-Tryptophan	0.34	0.33	0.23	0.25	0.20	0.28
L-Valine	0.2	0.33	0.10	0.24		0.13
Phytase	0.1	0.1	0.1	0.1	0.1	0.1
Total	1000	1000	1000	1000	1000	1000
Calculated Nutrient						
Dry Matter, %	87.19	88.26	87.27	88.18	87.43	87.97
NE Swine, Mcal/kg[3]	2.41	2.41	2.39	2.39	2.38	2.38
Crude Protein, %	18.14	18.14	15.75	15.63	14.45	14.05
Crude Fat, %	3.53	6.33	2.97	5.69	2.57	5.18
Crude Fiber, %	2.83	5.82	3.19	6.19	3.35	6.3
ADF[4], %	3.91	7.00	4.48	7.65	4.64	7.8
NDF[5], %	10.86	15.60	12.49	17.00	13.44	17.36
SID[6] Lysine, %	1.07	1.07	0.90	0.90	0.80	0.80
SID[6] Methionine, %	0.38	0.39	0.30	0.31	0.25	0.27
SID[6]Tryptophan, %	0.20	0.20	0.16	0.16	0.14	0.14
SID[6] Threonine, %	0.70	0.70	0.60	0.60	0.54	0.54
Available Ca, %	0.7	0.69	0.68	0.64	0.66	0.63
Available P, %	0.62	0.61	0.59	0.58	0.57	0.56
SID[6] Lys/NE Ratio	4.44	4.44	3.77	3.77	3.36	3.36

[1] The vitamin premix (DSM Nutritional Products Canada Inc., Ayr, ON) provided the following quantities of vitamins per kilogram of diets: vitamin A, 9,000 IU; vitamin D3, 1,167 IU; vitamin E, 90 IU; vitamin K, 2.7 mg; vitamin B1, 2.7 mg; vitamin B2, 7.4 mg;

vitamin B3, 33.0 mg; vitamin B5, 30.0 mg; vitamin B6, 3.0 mg; vitamin B7, 0.2 mg; vitamin B9, 1.1 mg; vitamin B12, 0.37 mg; vitamin C, 67 mg.

[2] The trace mineral premix (DSM Nutritional Products Canada Inc., Ayr, ON) provided the following quantities of minerals per kilogram of diets: Fe (ferrous sulphate), 140 mg; Cu (copper sulphate), 25 mg; I (calcium iodate), 1.0 mg; Se (selenium), 0.3 mg; Mn (manganous oxide), 75 mg; Zn (zinc oxide), 130 mg.

[3] Net energy.

[4] Neutral detergent fiber.

[5] Acid detergent fiber.

[6] Standardized ileal digestible.

Table 3.2 Analyzed nutrient composition of experimental diets, as-fed basis

Item	(25-55 kg)		(55-75 kg)		(75-110 kg)	
	Low fiber	High fiber	Low fiber	High fiber	Low fiber	High fiber
Dry Matter, %	88.80	88.88	89.34	89.77	88.90	89.94
Crude Protein, %	21.09	21.03	17.75	17.96	16.96	16.88
Crude Fat, %	2.53	4.80	2.73	5.00	2.32	4.11
Crude Fiber, %	2.30	4.55	2.58	5.59	2.49	5.65
ADF, %	3.97	8.23	5.36	10.41	4.89	9.16
NDF, %	12.17	16.30	10.95	17.69	11.72	16.71
NSP constituent sugars[2]						
Total NSP	6.76 (2.32)	10.83 (1.02)	7.43 (2.20)	11.23 (1.31)	7.87 (2.78)	12.59 (1.38)
Arabinose	1.47 (0.28)	1.53 (0.22)	1.55 (0.21)	1.55 (0.20)	1.55 (0.26)	1.66 (0.21)
Xylose	1.44 (0.30)	2.44 (0.11)	1.84 (0.47)	2.52 (0.09)	2.15 (0.52)	3.10 (0.09)
Mannose	ND	ND	ND	ND	ND	ND
Galactose	ND	0.65 (0.34)	ND	ND	ND	ND
Glucose	2.64 (1.55)	4.51 (0.25)	2.98 (1.46)	5.44 (0.97)	3.18 (1.83)	6.00 (0.92)
Uronic acids	1.22 (0.18)	1.71 (0.11)	1.06 (0.05)	1.72 (0.05)	1.00 (0.17)	1.83 (0.16)
Crude Ash, %	5.06	4.37	3.96	4.07	3.80	3.50
Acid Insoluble Ash, %	0.08	0.16	0.08	0.12	0.14	0.28
DE Swine, Mcal/kg	3.45	3.50	3.56	3.44	3.51	3.45
ME Swine, Mcal/kg	3.31	3.35	3.43	3.32	3.40	3.34

Non-Fiber Carbohydrate, %	60.23	55.67	62.91	58.62	64.81	61.29
Starch, %	39.33	34.33	46.48	37.37	48.80	40.45

Note: NDF, neutral detergent fiber; ADF, acid detergent fiber; DE, digestible energy; ME, metabolizable energy; ND, not detected.

Values in parentheses are for the soluble portion of non-starch polysaccharides (NSP) in the ingredient.

Digestible energy (DE) and metabolizable energy (ME) were estimated based on gross energy, assuming that DE equals 88% of GE and ME equals 96% of DE.

Table 3.3 Composition of soya hulls and oat hulls, as-fed basis, %

Item	Ingredients	
	Soya hulls	Oat hulls
NSP constituent sugars[2]		
Total NSP	61.53 (1.57)	52.82 (1.09)
Arabinose	2.98 (0.18)	2.18 (0.02)
Xylose	5.91 (0.15)	22.12 (0.37)
Mannose	4.47 (0.44)	ND
Galactose	2.31 (0.17)	ND
Glucose	33.87 (0.02)	26.68 (0.62)
Uronic acids	12.76 (0.68)	1.84 (0.08)

Note: NSP, non-starch polysaccharides; ND, not detected.

Values in parentheses are for the soluble portion of non-starch polysaccharides (NSP) in the ingredient.

3.3.3 Tissue collection

Eight pigs from the low- or high-fiber group were exposed to electroshock and exsanguination at a commercial slaughterhouse (Meatpacking Plant, Beausejour, MB, Canada). The abdomen was opened, and the small intestine was collected. After removing the digesta, the mid-jejunum (2.5 m proximal to the ileocecal junction) was washed in phosphate-buffered saline (PBS). The jejunal segments were carefully scraped with glass slides to collect mucosal samples and stored at -80 °C until further analysis.

3.3.4 Growth performance parameter measurement

The amount of diet added to each pen and the amount of feed remaining were recorded daily throughout the experiment, and the pigs were weighed at the end of each experimental phase. Average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were then calculated from the recorded feed intake and pig weights. The ADFI, ADG, and FCR were calculated using the following equation:

ADG (g/d) = (final BW of the pig - initial BW of the pig) / (number of feeding days).

ADFI (g/d) = (feed intake during feeding period) / (number of feeding days).

FCR=ADFI/ADG.

3.3.5 Biochemical analysis

Spectrophotometric measurement of total reduced glutathione and oxidized glutathione in the mucosa of the middle jejunum. Briefly, the mucosal layer was scraped from the jejunum, homogenized in PBS, and sonicated for 20 s. 10% sulfosalicylic acid (Sigma-Aldrich, Oakville, ON, Canada) was added to the suspension and then centrifuged at 5,000 × g for

10 min at 4 °C. Sodium phosphate buffer and 5,5′-Dithiobis (2-nitrobenzoic acid; Sigma-Aldrich, Oakville, ON, Canada) were added to the supernatant to test GSH levels. Sodium phosphate buffer, β-Nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt hydrate (Sigma Aldrich, Oakville, ON, Canada), GSH reductase (Sigma-Aldrich, Oakville, ON, Canada), and 5,5′-Dithio-bis (2-nitrobenzoic acid) were added to the supernatant to test GSSG levels. The concentration of GSH and GSSG were normalized to protein. The ratio of reduced glutathione to oxidized glutathione was measured as a redox potential indicator. Malondialdehyde levels in the mid-jejunal mucosa were determined using the thiobarbituric acid reactive substances assay.. Briefly, the mucosal layer was scraped from the jejunum, and the homogenate was prepared in PBS. 10% (w/v) phosphotungstic acid (Sigma-Aldrich, Oakville, ON, Canada) was added to the suspension and incubated for 10 min, then the mixture was centrifuged at 1,000 × g for 10 min at 4 °C and an aliquot of the supernatant was incubated with 0.67% (w/v) 2-thiobar-bituric acid (Sigma-Aldrich, Oakville, ON, Canada) for 1 h at 90 °C. Subsequently, the sample was centrifuged at 1000 × g for 10 min at 4 °C to collect the pellet, which was extracted with butanol for color to determine absorbance. The concentration of MDA was also normalized to protein.

3.3.6 Western immunoblotting analysis

The relative protein abundance of glutamate-cysteine ligase catalytic (Gclc) and modifier (Gclm) subunits and GS in mid-jejunal mucosa was measured by Western blotting analysis. Total proteins (20 to 60 μg) were taken in 10% SDS-polyacrylamide gels for electrophoretic separation and then transferred to a nitrocellulose membrane. For

immunoblotting, the membranes were first blocked for 1 h at room temperature with 5% skim milk powder in tris-buffered saline containing 0.05% Tween-20. Total mucosal proteins of the jejunum were then examined at 4 °C using antibodies against rabbit anti-Gclm monoclonal (1:1,000, Abcam, Inc., Toronto, Canada), rabbit anti-Gclc monoclonal (1:1,000, Abcam), or rabbit anti-glutathione synthetase monoclonal (1:1,000, Abcam). HRP-conjugated anti-rabbit IgG antibody (Cell Signaling Technology, Danvers, MA) was used as a secondary antibody (1:2,000). β-Actin (1:5,000, Cell Signaling Technology) was used as an internal reference. Data are expressed as mean ± SEM (n = 6 to 8).

3.3.7 Real-time PCR

Total RNA was isolated from jejunal tissue using TRIzol reagent (Invitrogen, Carlsbad, CA). All primers were designed using Primer-Blast based on porcine gene sequences (Table 3.4). Gclc, Gclm, and GS mRNA were analyzed by real-time PCR using the iQ5 real-time PCR detection system (Bio-Rad, Mississauga, ON, Canada). β-actin was used as a housekeeping gene to normalize the transcript levels of the target genes. A comparative threshold cycle (Ct) approach was used to calculate the quantitative expression levels of target genes relative to β-actin (Lupberger et al., 2002). Data are expressed as relative values for pigs fed a high-fiber diet.

Table 3.4 Forward and reverse primer sequences for Real-time PCR

Gene	Accession no.	Primer sequence, 5'-3'	References
β-actin	NM_001172909.1	F: CTGCGGCATCCACGAAACT	(Yin et al., 2014)
		R: AGGGCCGTGATCTCCTTCTG	
Gclc	XM_003482164.4	F: GGCGACGAGGTGGAATACAT	(Yan et al., 2020)
		R: GTTTGGGTTTGTCCTTTCCCC	
Gclm	XM_001926378.4	F: ACAATACAACGGTTCAGGTGAGT	(Deng et al., 2021)
		R: GCCTGTAAAATGTGTCATTGAGG	
GS	NM_001244625.1	F: AAGAAGCTGCCAAGATCCTC	(Bausys et al., 2021)
		R: ATTCTCTATGGCACGCTGGT	

Note: *β-actin*, beta-actin; *Gclc*, glutamate-cysteine ligase catalytic subunit; *Gclm*, glutamate-cysteine ligase modified subunit; *GS*, glutathione synthetase.

3.3.8 Statistical analysis

Growth performance and oxidative stress data were analyzed using SAS (version 9.4; SAS Inst. Inc., Cary, NC) with pen and individual pig as experimental unit, respectively. All data were assessed for normality with PROC UNIVARIATE, and outliers were tested using the studentized residual analysis. The growth performance data were analyzed as repeated measures with treatment, phase, and treatment × phase interactions included in the model. Results of growth performance analyses are reported as least squares means. Student's t-tests were used to assess the effect of fiber on overall ADG, ADFI, FCR, and oxidative stress data. These results were expressed as means and SEM. Differences were considered significant at $P < 0.05$.

3.4 Results

3.4.1 The chemical and fiber composition of the fiber sources and experimental diets

Although the NSP of soya hulls and oat hulls are water insoluble, the fiber structure of both hulls is different. Hemicellulose in soya hulls and oat hulls consisted predominantly of xylose residues. The content of glucose residues was higher in soya hulls than in oat hulls, whereas the concentration of soluble glucose residues in oat hulls was higher than in soya hulls. Soybean hulls had a higher content of insoluble NSP and a higher content of soluble NSP and were characterized by a higher pectin content, as indicated by the higher uronic acids than oat hulls. Oat hulls were rich in insoluble and soluble mannose residues (Table 3.3).

By analyzing the NSP of the experimental diets, the main fibrous components of the dietary treatments were similar (Table 3.2). The high-fiber diets were rich in all insoluble NSP

components, especially insoluble xylose, glucose, and uronic acids residues. On the contrary, the characteristics of the low-fiber diets was higher in all soluble NSP components than in high-fiber diets.

3.4.2 Feed intake and growth performance

We examined the feed intake and growth performance in pigs fed a high- or low-fiber diet. As shown in Table 3.5, interactions between treatment and phase for BW and FCR were significant. Pigs fed a high-fiber diet had heavier body weights on day 70 than pigs fed a low-fiber diet. No interactions were found between treatment and phase for ADG and ADFI. Throughout the feeding period, pigs fed a high-fiber diet had higher ADG than those fed a low-fiber diet. From days 35 to 42, FCR increased in the low-fiber fed group compared to the high-fiber fed group, but there was no significant difference in FCR between the two groups in the other two trial phases. There was no significant difference in FCR and ADFI between the two groups from days 0 to 70.

Table 3.5 Effects of high- and low-fiber diets on growth performance of growing-finishing pigs

Items	Treatment (Trt)		Pooled	*P* Value		
	High	Low	SEM	Trt	Phase	Trt ×Phase
BW, kg						
Day0	27.7	27.69				
Day35	66.81	63.07	1.19	0.006	<0.001	0.017
Day42	75.92	71.88				
Day70	109.16[a]	102.82[b]				
ADG, kg/d						
Day0 to 35	1.12	1.05				
Day35 to 42	1.30	1.09	0.04	0.017	0.076	0.210
Day42 to 70	1.19	1.16				
Day0 to 70	1.16[a]	1.10[b]	0.02		0.027	
ADFI, kg						
Day0 to 35	2.28	2.15				
Day35 to 42	2.92	2.98	0.11	0.631	<0.001	0.405
Day42 to 70	3.26	3.17				
Day0 to 70	2.09	2.02	0.07		0.460	
FCR						
Day0 to 35	2.04	2.08				
Day35 to 42	2.29[b]	2.71[a]	0.11	0.071	<0.001	0.019

Day42 to 70	2.75	2.74		
Day0 to 70	2.35	2.42	0.06	0.401

Results are expressed as least squares means (n = 10 for each group). [a,b] Means in the same row with different superscript differ ($P < 0.05$). BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; Trt, treatment.

3.4.4 Oxidative stress biomarkers in the intestine

We examined the oxidative stress biomarkers (MDA) in the intestine of pigs fed a high- or low-fiber diet. The level of malondialdehyde was significantly increased in the jejunum of pigs fed a low-fiber diet, indicating increased oxidative stress (Figure 3.1a). Low-fiber diet resulted in a significant increase in the levels of GSH and GSSG in the jejunum (Figure 3.1b, c). However, there was no significant difference in the ratio of GSH to GSSG between the two groups (Figure 3.1d).

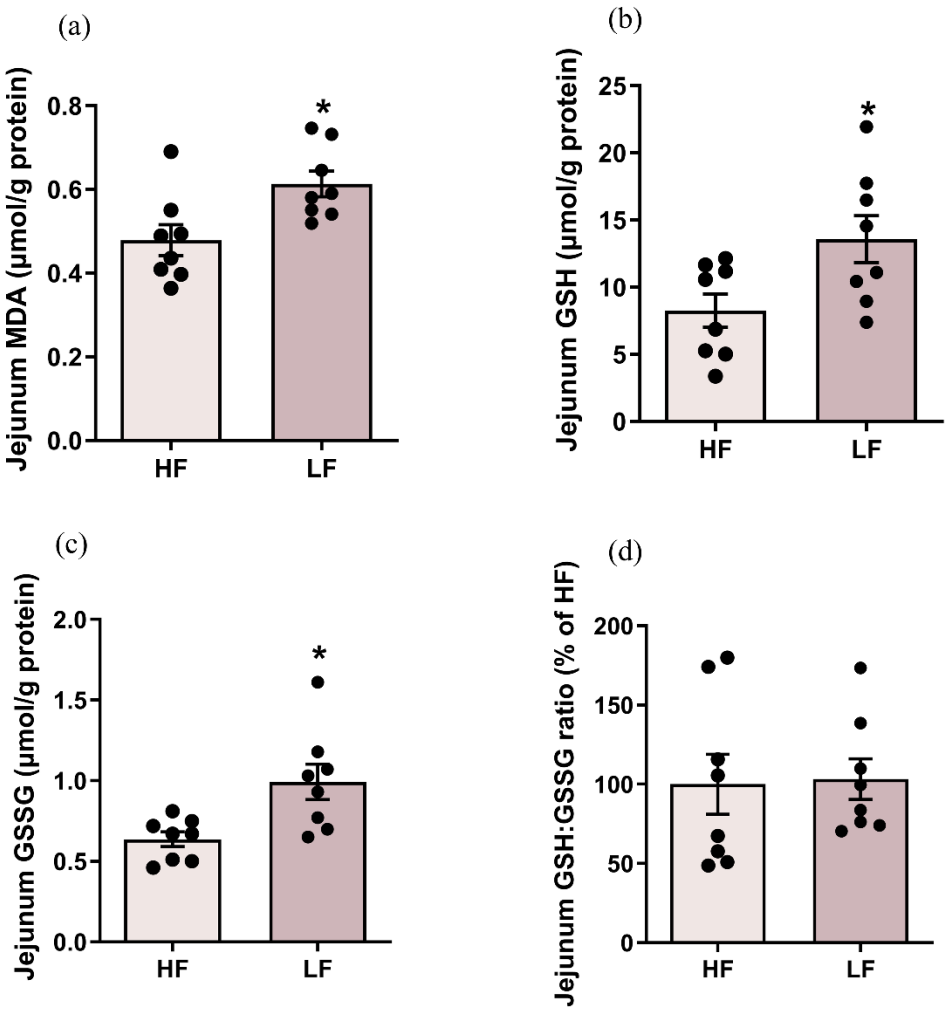

Figure 3.1 Effect of fiber on glutathione levels and lipid peroxidation in the jejunum

Jejunal malondialdehyde (MDA) levels (a), reduced glutathione (GSH) (b), oxidized glutathione (GSSG) (c), and a ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) (d) were measured in pigs fed a high-fiber (HF) or low-fiber (LF) diet. Results are expressed as mean ± SEM (n = 8 for each group). *$P < 0.05$ when compared with the value obtained from the HF group.

3.4.5 Expression of antioxidant enzymes and transcription factors in the intestine

To investigate the reasons for the changes in glutathione levels, we examined the expression of the enzymes responsible for glutathione synthesis in the intestine. There was a significant increase in Gclc mRNA and protein levels in the jejunum of the low-fiber group (Figure 3.2a and b). However, there was no significant difference in the mRNA and protein levels of Gclm and glutathione synthetase between the low-fiber and high-fiber fed groups (Figure 2c-f). Glutamate-cysteine ligase is a rate-limiting enzyme in glutathione synthesis. Increased expression of its catalytic subunit (Gclc) might contribute to an elevation of GSH in the jejunum of pigs fed a low-fiber diet.

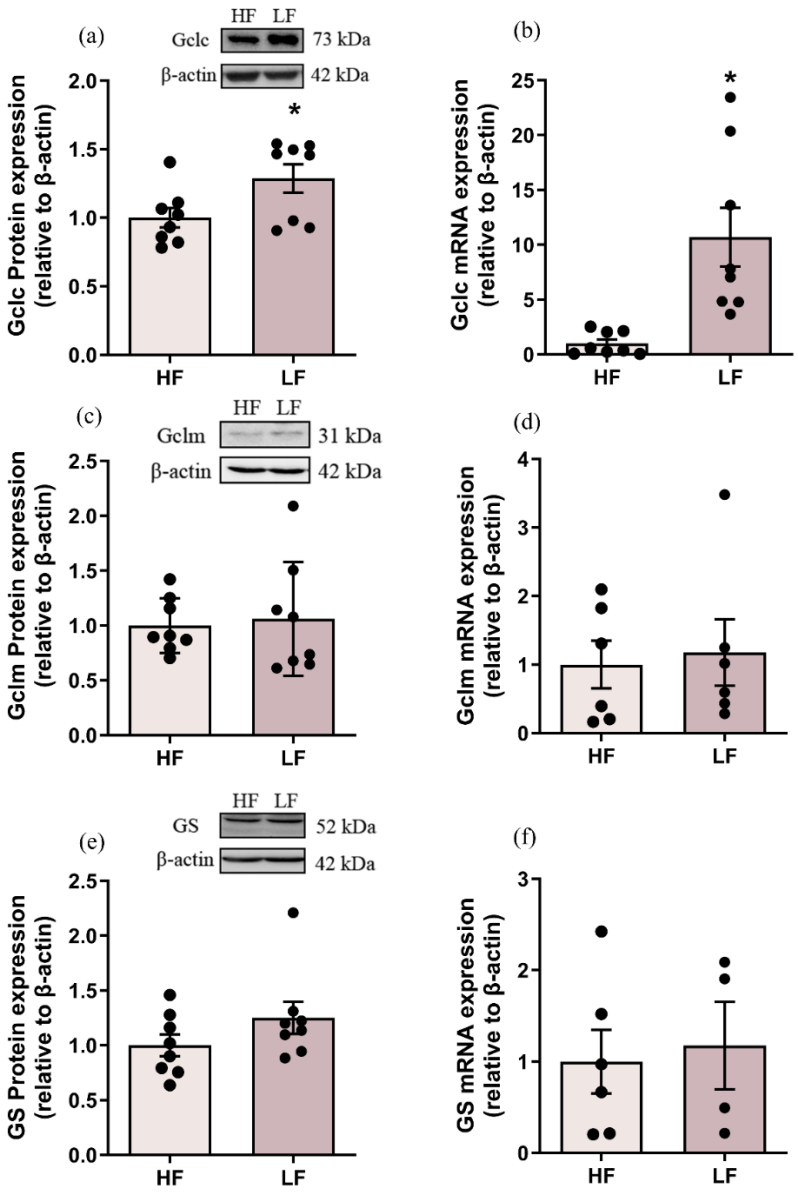

Figure 3.2 Expression of glutathione synthesizing enzymes in the jejunum

Jejunal glutamate-cysteine ligase catalytic subunit (Gclc) protein (a) and mRNA (b), glutamate-cysteine ligase modifier subunit (Gclm) protein (c) and mRNA (d), glutathione synthetase (GS) protein (e) and mRNA (f) were measured in pigs fed a high-fiber (HF) or low-fiber (LF) diet. Results are expressed as mean ± SEM (n = 4 to 8 for each group). *P < 0.05 when compared with the value obtained from the HF group.

3.5 Discussion

In the present study, feeding a high-fiber diet to growing pigs significantly increased ADG and final body weight. Feeding a low-fiber diet increased oxidative stress biomarkers in the jejunum, indicating the presence of oxidative stress.

Although soybean hulls and oat hulls were added to the high-fiber diets to increase the total NSP content in the diets, both the high- and low-fiber diets were characterized by the presence of high levels of arabinoxylan, as indicated by high levels of insoluble arabinose and xylose residues. In addition, the low-fiber diets were characterized by soluble glucose residues due to the high levels of β-glucan in various cereals such as corn, wheat, and barley (Hu et al., 2015). Soybean hulls contain xylose residues in a β-1,4 mode. The xylose residues in soybean hulls are linked to the O-3 position of homogalacturonan (Mohnen, 2008). The homogalacturonan residues are interrupted by l-rhamnose residues and various side chains (Karr-Lilienthal et al., 2005). Other studies have reported that β-1,4-linked xylose may be attached to rhamnose-I (Mohnen, 2008). This may contribute to the high levels of xylose and uronic acid residues in high-fiber diets. High-fiber diets also contained high levels of cellulose, contributing to increased levels of insoluble glucose-based polysaccharides.

Although low- and high-fiber diets had the same primary nutrient composition, there was a significant difference in growth performance between low- and high-fiber-fed groups. In the current study, it was observed that high-fiber supplementation increased body weight on day 70 as well as increased average daily gain during the whole experimental period.

This might be due to the healthier intestinal tract of pigs fed a high-fiber diet (Williams et al., 2001; Montagne et al., 2003). Fiber can be fermented in the hindgut by intestinal microorganisms to produce short-chain fatty acids (Jha et al., 2010; Jha and Leterme, 2012), which promotes the growth of beneficial microorganisms and inhibits the survival of harmful bacteria in the intestine (Pieper et al., 2008). Similarly, Kim et al. (2008) and González-Ortiz et al. (2014) also reported the ability of oat hulls or soya hulls against *E. coli* K88. The ADFI of pigs is influenced by their body weight and health status, the palatability of the diet, and the level of nutrition. Previous studies have reported that weaned piglets require increased feed intake to obtain adequate nutritional requirements due to the ability of insoluble fiber to reduce the residence time of the diet in the intestine, affecting intestinal absorption of nutrients from the diet (Wenk, 2001; Gerritsen et al., 2012). In addition, Hopwood et al. (2004) reported that beet pulp, which contains mainly soluble fiber, can reduce daily feed intake in weaned piglets by increasing the time of digestive materials in the digestive tract. These inconsistent results are related to the different sources of fiber supplementation in the diets (Zhao et al., 2018). In our study, the addition of oat hulls and soybean hulls, mainly insoluble fiber (Jaworski and Stein, 2017; Ndou et al., 2017), to the diets of growing pigs did not affect the ADFI of growing pigs. The possible reason is that the intestine of growing pigs is fully developed and absorbs nutrients from the diet more efficiently than weaned piglets (Ndou et al., 2017). Overall, our results suggested that a high-fiber diet could promote growth performance in growing-finishing pigs. The gastrointestinal tract is the largest organ in the body. It is the site of digestion and absorption of dietary nutrients (Jha et al., 2019). Intestinal oxidative stress may lead to reduced intestinal villus height and broken tight junction proteins, which in

turn lead to compromised digestive and absorptive function of the intestine and deterioration of intestinal health, and ultimately reduced growth performance of piglets (Yuan et al., 2007). Our results also showed that the growth performance of pigs in the low-fiber group was significantly lower than that of pigs in the high-fiber group. Water-insoluble fibers such as soybean hulls and oat byproducts may have considerable antioxidant potential and benefit the pig organism (Sobotka et al., 2012; Liu et al., 2022a). High insoluble fiber intake in the late gestation period of sows can improve redox status by reducing serum MDA levels (Liu et al., 2020d). MDA is the most used biomarker for identifying lipid peroxidation. A novel observation of the present study was that fiber could protect the jejunum from oxidative stress. In the present study, we found that the MDA level in the jejunum of the low-fiber group was significantly higher than that in the high-fiber group, reflecting oxidative stress in the jejunum of growing-finishing pigs fed a low-fiber diet. Glutathione acts as a major endogenous antioxidant to counteract oxidative stress. Increasing insoluble water fiber in the diet of rats could decrease serum GSSG levels (Wang et al., 2011). In the present study, a low-fiber diet resulted in a significant increase in GSH and GSSG levels in the jejunum, suggesting that pigs fed a low-fiber diet might need to produce more GSH to cope with oxidative stress in the intestine.

Fewer studies have been conducted on the effects of fiber on the glutathione synthesizing enzymes in the intestine. The first step in GSH biosynthesis is the rate-limiting step, catalyzed by glutamate-cysteine ligase (GCL, also known as γ-glutamylcysteine synthase). The GCL enzyme consists of a catalytic subunit (Gclc) and a modifier subunit (Gclm), which dissociate under reducing conditions (Seelig et al., 1984; Yan and Meister, 1990;

Huang et al., 1993). In the present study, a low-fiber diet resulted in a significant increase in the mRNA and protein expression of the jejunal Gclc, an enzyme that catalyzes the rate-limiting reaction for glutathione synthesis. Increased Gclc expression might lead to an increase in GSH production. Our results also showed increased GSH levels in the jejunum of pigs fed a low-fiber diet. These results suggested that the intestine of growing pigs fed a low-fiber diet might have experienced oxidative stress, which upregulated GSH synthesis. However, increased GSH synthesis was insufficient to attenuate oxidative stress as the level of MDA, a biomarker of lipid peroxidation, remained elevated in the intestine of pigs with a low-fiber diet.

3.6 Conclusions

The present study has demonstrated that a high-fiber diet could improve growth performance and maintain gut health, as indicated by low intestinal oxidative stress (decreased MDA level) in growing-finishing pigs. In addition, pigs in the low-fiber group responded to oxidative stress by producing GSH by activating glutamate-cysteine ligase. These findings indicate the potential application of a high-fiber diet as a safe and effective nutritional strategy to maintain gut health in pigs. Our results suggest that the fiber content in diets may contribute to the health of the animal intestine and improve animal welfare. Further studies are needed to investigate the mechanisms by which fibers protect the intestine from oxidative stress.

Transition statement

In Study 1, we observed that dietary interventions significantly influenced intestinal antioxidant capacity. Specifically, a high-fiber diet could enhance the overall antioxidant response in the intestine, hence mitigating oxidative stress and reducing the accumulation of oxidative damage markers. In contrast, a low-fiber diet was associated with a decrease in antioxidant capacity and increased oxidative stress in the intestine. These results highlight the critical role of dietary fiber in modulating intestinal redox balance and the potential of dietary strategies to mitigate oxidative stress-related injury. However, while we have established the role of dietary modulation in endogenous antioxidant defense, our understanding of oxidative stress mechanisms under conditions of external pathogenic challenge remains limited. To address this gap, we conducted Study 2. We employed ETEC infection as an exogenous stress inducer. ETEC is a prevalent pathogen known to cause diarrhea and intestinal injury in weaned piglets by disrupting the intestinal barrier, inducing inflammatory responses, and increasing oxidative stress (Sun and Kim, 2017). ETEC infection is a critical model for studying intestinal oxidative stress as it leads to substantial accumulation of reactive oxygen species and impairs antioxidant defense systems (Wang et al., 2024b). Additionally, ETEC infection is often accompanied by decreased expression of tight junction proteins, resulting in increased intestinal permeability, which exacerbates oxidative stress and inflammation (Lodemann et al., 2017). The objective of Study 2 was to investigate how ETEC-induced oxidative stress impacts intestinal function and whether restoration of antioxidant defense mechanisms can mitigate these detrimental effects. We aimed to explore potential therapeutic targets that affect intestinal health during pathogenic challenges.

CHAPTER FOUR: MANUSCRIPT II

Regulation of oxidative stress in the intestine of piglets after enterotoxigenic *Escherichia coli* (ETEC) infection

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4.1 Abstract

Enterotoxigenic *Escherichia coli* (ETEC) is recognized globally as a major gastrointestinal pathogen that impairs intestinal function. ETEC infection can lead to oxidative stress and disruption of intestinal integrity. The present study investigated the mechanism of increased oxidative stress and whether restoration of antioxidant defense could improve intestinal integrity in a piglet model with ETEC infection. Weaned piglets were divided into three groups: control, ETEC-infection and ETEC-infection with antibiotic supplementation. The infection caused a significant elevation of serum diamine oxidase activity and D-lactate levels coupled with a reduced intestinal (mid-jejunum) tight junction protein expression, suggesting increased intestinal permeability and impaired gut function. The infection also inhibited nuclear factor erythroid 2-related factor 2 (Nrf2) activation, decreased the expression of glutathione synthesizing enzymes, superoxide dismutase-1 (SOD1), and heme oxygenase-1 (HO-1) in the intestine. This led to a decreased antioxidant glutathione level and an increased lipid peroxidation in the intestine and serum, indicating oxidative stress. The infection stimulated the expression of pro-inflammatory cytokines (IL-6, TNF-α). Antibiotic supplementation attenuated oxidative stress, in part, through restoration of glutathione levels and antioxidant enzyme expression in the intestine. Such treatment enhanced tight junction protein expression and improved intestinal function. Furthermore, induction of oxidative stress in Caco2 cells by hydrogen peroxide inhibited tight junction protein expression and stimulated inflammatory cytokine expression. Glutathione supplementation effectively attenuated oxidative stress and restored tight junction protein expression. These results suggest that downregulation of Nrf2 activation

may weaken antioxidant defense and increase oxidative stress in the intestine. Mitigation of oxidative stress can improve intestinal function after infection.

Key words: Oxidative stress, Intestine, Glutathione, Piglets, Enterotoxigenic *Escherichia coli*

4.2 Introduction

Oxidative stress occurs when there is an imbalance between the production of ROS and its removal by the antioxidant defense system. ROS at elevated levels can cause cellular injury, including lipid peroxidation, protein denaturation, and DNA damage (Sies and Jones, 2020). The gastrointestinal tract is more susceptible to oxidative stress as it is frequently exposed to food components and toxins (endotoxins and mycotoxins) that can trigger ROS accumulation. The post-weaning period is critical for gastrointestinal health in humans and animals, which has a significant influence on growth and health (Arthi and Schneider, 2021; Kim et al., 2022a). The maintenance of the epithelial barrier integrity is critical to intestinal function (Tao et al., 2019; Ghosh et al., 2021; Duarte and Kim, 2022). Damage to the epithelial barrier leads to increased intestinal permeability, allowing bacterial endotoxins to enter the bloodstream, which can cause serious illness (Arbizu et al., 2020; Martel et al., 2022). Infection-induced oxidative stress causes further damage to the tight junction of the intestinal epithelium, thus exacerbating the barrier dysfunction (Cao et al., 2020).

E. coli is a type of gram-negative bacteria that resides naturally in the intestine of humans and animals (Foster-Nyarko and Pallen, 2022; Pokharel et al., 2023). Among its various strains, enterotoxigenic *Escherichia coli* F4 is known for its pathogenicity in pigs causing severe diarrhea (Sun and Kim, 2017; Li et al., 2021b). ETEC F4 can disrupt the intestinal epithelial barrier by decreasing the expression of tight junction proteins such as ZO-1 and occludin in the intestine, causing increased intestinal permeability (Duan et al., 2022). A recent study suggests that ETEC infection can induce oxidative stress in the intestine (Jiménez et al., 2020). In piglets, the increased intestinal permeability in the post-weaning

period can facilitate the translocation of toxins and pathogens from the lumen of gut to the circulation, causing systemic oxidative stress and inflammation (Tang et al., 2022). Increased oxidative stress and inflammation can exacerbate gut damage after infection (Pickard et al., 2017; Lin et al., 2021). Although *E. coli* infection can affect the function of the intestinal barrier, the role of oxidative stress in intestinal health is not well understood.

ROS are highly reactive free radicals that can elicit inflammation and tissue damage, which is associated with many diseases (Martemucci et al., 2022). The production of ROS at low levels is a physiological process that occurs during normal cellular metabolism (Yang and Lian, 2020). The body has natural defense mechanisms against oxidative stress, including enzymes and antioxidants that metabolize or neutralize ROS (Hajam et al., 2022). Nuclear factor erythroid 2-related factor 2 is a key transcription factor that regulates the gene expression of enzymes involved in antioxidant defense (Liu et al., 2022b). Under unstressed conditions, Nrf2 protein is retained in the cytoplasm via binding to Kelch-like ECH-associated protein 1. In response to oxidative stress, Nrf2 is disassociated from Kelch-like ECH-associated protein 1 and translocated into the nucleus where it binds the antioxidant response elements in the promoter region of genes encoding enzymes that are involved in antioxidant defense mechanisms (Chen et al., 2024a). Activation of Nrf2 enhances the expression of antioxidant enzymes including SOD1, HO-1 and glutathione synthesizing enzymes.

Glutathione is a tripeptide containing thiol, a potent antioxidant that plays an essential role in fighting oxidative stress (Chai and Mieyal, 2023). Glutathione is synthesized through

two sequential reactions. Initially, γ-glutamylcysteine is produced by glutamate-cysteine ligase that contains a catalytic subunit (Gclc) and a modifier subunit (Gclm). Subsequently, glycine is added to form glutathione in a reaction catalyzed by glutathione synthetase (Lu, 2013). The effectiveness of glutathione relies on its reduced form (GSH) in neutralizing ROS, which is subsequently oxidized to GSSG (Sreekumar et al., 2021). Any disturbance in glutathione levels or an imbalance in the GSH/GSSG ratio is indicative of oxidative stress (Circu and Aw, 2012). Little information is available regarding the impact of *E. coli* infection on glutathione biosynthesis in the intestine. Although it was reported that ETEC infection could induce oxidative stress in the intestine of mice with a decreased GSH level (Tang et al., 2019), the molecular mechanism is not clear. Antibiotics have traditionally been used in animal production to treat severe bacterial infection, especially those caused by pathogens such as ETEC (Low et al., 2021). In the present study, we investigated the effect of ETEC F4 infection on intestinal oxidative stress and the mechanisms involved, and whether attenuation of oxidative stress could improve intestinal integrity in a piglet model.

4.3 Materials and methods

4.3.1 Animal model

Weaned piglets are more susceptible to enteric infection causing intestinal dysfunction with impaired gut barrier integrity and nutrient absorption (Tang et al., 2022). In the present study, the selection of piglets that were susceptible to enterotoxigenic *Escherichia coli* F4 infection was based on the identification of MUC4 gene present in the piglets (Sterndale et al., 2019a; Choi et al., 2020).The tail DNA samples were collected from piglets (TN Tempo

× TN70) on the third day post-farrowing and allele differentiation was conducted using FastDigest XbaI digestion (Thermo Fisher Scientific, Waltham, MA, USA) and 2 % agarose gel electrophoresis for the identification of MUC4 gene. The piglets with the susceptible allele and average body weight of 6.5 kg were selected. A total of 18 weaned piglets (28 days old) that were susceptible to ETEC F4 were randomly divided into three groups: Group 1 (control), Group 2 (*E. coli* infection), and Group 3 (*E. coli* infection received antibiotic avilamycin). Each group had 6 piglets (3 male and 3 female). Piglets in Group 1 and 2 were fed a corn soybean meal diet, a basal diet designed to meet the NRC guidelines (2012) for piglets weighing between 6 and 10 kg. Piglets in Group 3 were fed a corn-soybean meal diet supplemented with avilamycin (800 mg Surmax (10 % premix) /kg diet, Elanco, AB, Canada). On day 7, Group 2 and Group 3 were infected with *E. coli* by an oral gavage (5 mL of ETEC F4, 1×10^6 CFU/mL, Veterinary Diagnostic Services Laboratory, MB, Canada) (Choi et al., 2020). Five days after infection, blood samples were collected from the jugular vein and the serum was separated by centrifugation of blood at 4000 ×g for 10 min at 4 ◦C. The small intestine samples were collected after euthanasia. In brief, the mid-jejunum (approximately 2.0 m away from the ileocecal junction) was collected and the digesta was removed. The jejunal segment (5 cm) was cut open longitudinally, rinsed in phosphate-buffered saline and the mucosal epithelium was carefully scraped with a glass slide (Jin et al., 2022). The serum and intestinal samples were stored at 80 ◦C until further analysis. All piglets were cared for according to the Canadian Council on Animal Care guidelines, and all procedures were approved by the University of Manitoba Animal Care Committee.

4.3.2 Biochemical analysis

Serum diamine oxidase activity and D-lactate levels were quantified using commercial kits (Abcam Inc., Toronto, ON, Canada). Malondialdehyde in the intestine, serum, and Caco2 cells were determined using the thiobarbituric acid reactive substances method (Prathapasinghe et al., 2007; Jin et al., 2022). The glutathione in the reduced form (GSH) and the oxidized form (GSSG) were measured using spectrophotometry (Prathapasinghe et al., 2007; Jin et al., 2022)

4.3.3 Cell culture

Caco2, human colon cancer cells (ATCC, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium with 10 % fetal bovine serum, incubated at 37 ◦C in a humidified 5 % CO_2 atmosphere according to the ATTC's instruction. Cells were seeded at a density of 1×10^5 cells/mL in culture dishes. Caco2 cells are widely used as an intestinal cell model for studies on the regulation of epithelial permeability, nutrient absorption, oxidative and inflammatory response. The differentiated cells resemble many characteristics of enterocytes in the small intestine such as the formation of tight junction proteins, expression of enzymes and transporters for nutrient absorption (Vachon and Beaulieu, 1992). To induce oxidative stress, cells were incubated with hydrogen peroxide (200, 400, 800 μM) for 4 h. In other sets of experiments, cells were preincubated with glutathione (0.5, 1 mM) followed by incubation with hydrogen peroxide for 4 h. At the end of incubation, the intracellular oxidative stress biomarkers, expression of tight junction proteins and inflammatory cytokines were measured. To examine cell viability, water-

soluble tetrazolium (WST-1) salt was used according to the manufacturer's instructions (Sigma-Aldrich, Taufkirchen, Germany).

4.3.4 Quantitative polymerase chain reaction (PCR) analysis

Total mRNAs were prepared from the intestine and Caco2 cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After converting RNA to cDNA by reverse transcription, the mRNA levels of individual genes were measured by a quantitative PCR analysis using the StepOnePlusTM Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) as described in previous studies (Au-Yeung et al., 2023). The gene expression was normalized to that of the housekeeping gene β-actin. The primers specific to Gclc, Gclm, IL-1β, ZO-1, GS, occludin, TNF-α, Nrf2, HO-1, SOD1, IL-6, and β-actin were designed using the Primer-Blast website (Table 4.1).

Table 4.1 Primer sequences used for real-time PCR

Genes	Primer sequences (5'-3')	Accession Number	Size (bp)
Swine			
Gclc	F: GTCCAGTTGGTCCTGTCTGG R: CGGGAGTCCCTTCGATCATG	XM_021098556.1	127
Gclm	F: TTGGAGCAGCTGTACCAGTG R: GAGCTTCCTGGAAACTCGCT	XM_001926378.4	175
GS	F: GTGCTCAAGCCCCAGAGA R: ATGAGGCTCTCTCCTCACTGTC	NM_001244625.1	119
SOD1	F: GTACCAGTGCAGGTCCTCAC R: TTTGCCAGCAGTCACATTGC	NM_001190422.1	104
HO-1	F: GCTGAGAATGCCGAGTTCAT R: GCTGAGAATGCCGAGTTCAT	NM_001004027.1	142
ZO-1	F: GATCCTGACCCGGTGTCTGA R: TTGGTGGGTTTGGTGGGTTG	XM_021098896.1	200
Occludin	F: GAGAGAGTGGACAGCCCCAT R: TGCTGCTGTAATGAGGCTGC	NM_001163647.2	163
TNF-α	F: TTCCAGCTGGCCCCTTGAGC R: GGCATTGGCATACCCAC	NM_214022.1	143
IL-6	F: AAGGTGATGCCACCTCAGAC R: TCTGCCAGTACCTCCTTGCT	NM_001252429.1	151
IL-1β	F: ACATGCTGAAGGCTCTCCAC R: CAGGGTGGGCGTGTTATCTT	NM_214055.1	170

β-actin	F: CTGCGGCATCCACGAAACT R: AGGGCCGTGATCTCCTTCTG	NM_001172909.1	147
Human			
ZO-1	F: AACATACAGTGACGCTTCACA R: CACTATTGACGTTTCCCCACTC	NM_003257.5	105
Occludin	F: GACTATGTGGAAAGAGTTGAC R: GCTGCTGTAACGAG	NM_001410743.1	171
TNF-α	F: CCGATGGCCACAGATGTCTT R: AAGGTGGTGGCGATGGATTT	NM_001077654.3	98
IL-6	F: TACCCCCAGGAGAAGATTCC R: AGTGCCTCTTTGCTGCTTTC	NM_001371096.1	166
β-actin	F: CACCAACTGGGACGACAT R: ACAGCCTGGATAGCAACG	NM_001101.5	189

4.3.5 Western immunoblotting analysis

The protein levels of enzymes, transcription factors and tight junction proteins were determined using Western immunoblotting analysis. These included glutathione synthesizing enzymes, SOD1, HO- 1, Nrf2, occludin, and ZO-1. Briefly, proteins prepared from the intestine (40-80 µg) were separated by 8-12 % SDS-PAGE (Wijerathne et al., 2022). The separated proteins were transferred to nitrocellulose membranes and incubated with specific primary antibodies followed by incubation with secondary antibodies (Table 4.2). The β-actin and Lamin B1 were used to ensure equal loading of proteins from individual samples.

Table 4.2 Primary antibodies for Western immunoblotting analysis

Protein	Antibody	Dilution factor	Source	Catalog number
Gclc	Rabbit anti-GCLC polyclonal	1:1000	Abcam	ab41463
Gclm	Rabbit anti-GCLM polyclonal	1:1000	Abcam	ab118974
GS	Rabbit anti-GS polyclonal	1:1000	Abcam	ab91591
SOD1	Rabbit anti-SOD1 polyclonal	1:1000	Abcam	ab13498
HO-1	Rabbit anti-HO-1 monoclonal	1:1000	Abcam	ab189491
Nrf2	Rabbit anti-Nrf2 monoclonal	1:1000	Abcam	ab62352
ZO-1	Rabbit anti-ZO-1 polyclonal	1:1000	Invitrogen	61-7300
Occludin	Rabbit anti-Occludin polyclonal	1:1000	Invitrogen	71-1500
β-actin	Rabbit anti-β-actin monoclonal	1:1000	Cell signaling	4970s
Lamin B1	Rabbit anti- Lamin B1 polyclonal	1:1000	Abcam	ab16048

4.3.6 Statistical analysis

Data analysis was performed using SAS software (Version 9.4; SAS Institute Inc., Cary, NC, USA). The normality of the data distribution was assessed using the PROC UNIVARIATE procedure followed by using unpaired two-tailed *t*-test or one-way ANOVA. Results were reported as mean ± SD and were considered statistically significant if the *P* value was below 0.05.

4.4 Results

4.4.1 ETEC F4 infection impaired intestinal integrity and increased oxidative stress biomarkers in the serum

The increased intestinal permeability can be characterized by elevated diamine oxidase (DAO) activity in the serum (Yu et al., 2021). ETEC F4 infection led to an elevation of DAO activity and D-lactate (bacterial byproduct) levels in the serum compared to that in the control group (Figure 4.1 A, B), suggesting that the intestinal permeability might have been compromised. Antibiotic supplementation significantly reduced serum DAO activity and D-lactate levels in piglets with ETEC F4 infection (Figure 4.11A, B). ETEC F4 infection also decreased the serum GSH level and a GSH/GSSG ratio in the piglets (Figure 4.1 C, D, E). Antibiotic supplementation restored the serum GSH level and a GSH/GSSG ratio (Figure 4.1C, D, E). The serum lipid peroxide malondialdehyde (MDA) level was significantly elevated in piglets after ETEC F4 infection, while antibiotic supplementation reduced MDA level in the serum (Figure 4.1 F). These results suggested that ETEC F4 infection increased intestinal permeability and oxidative stress biomarkers in the circulation.

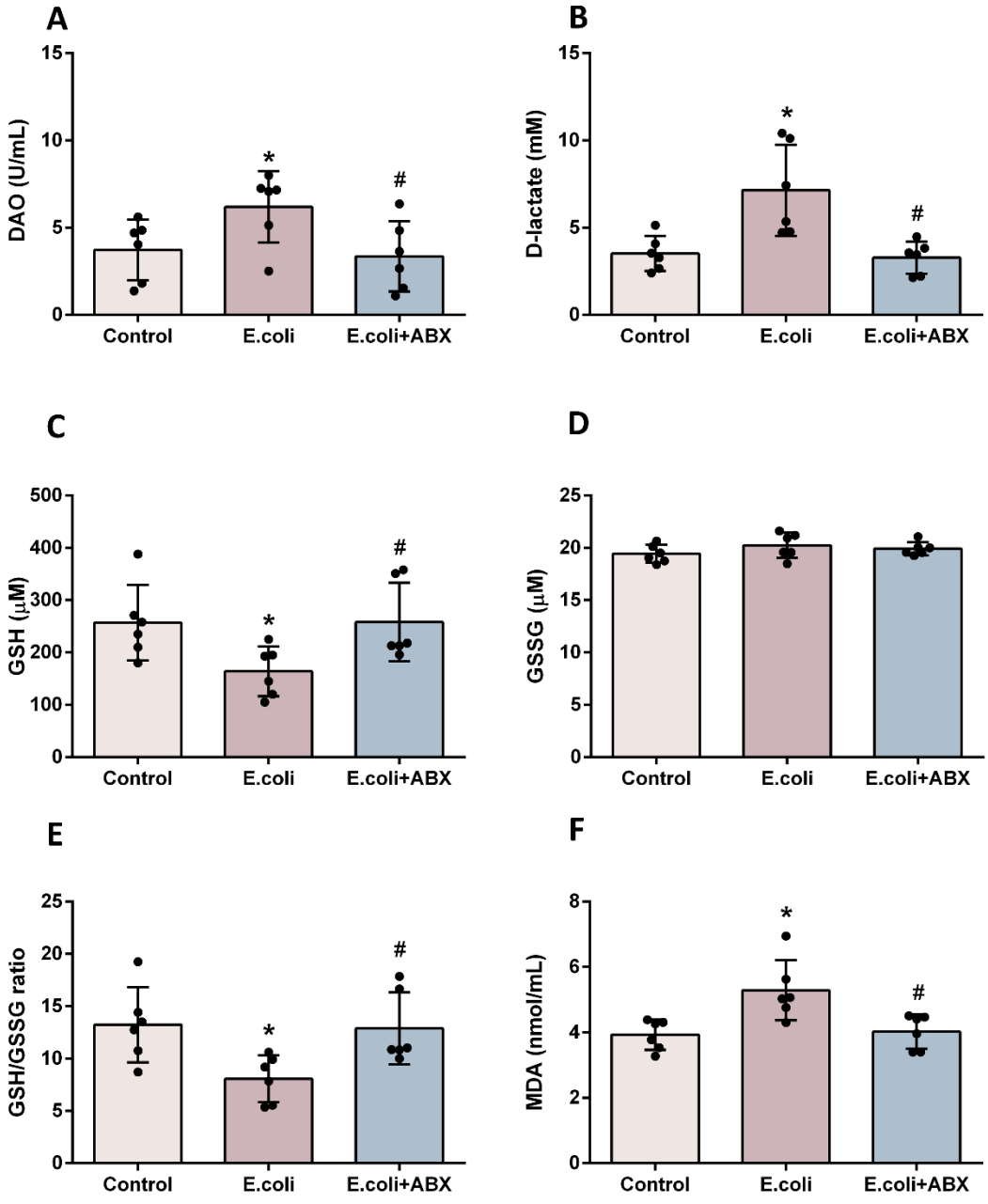

Figure 4.1 Serum parameters for oxidative stress and intestine integrity

Serum samples were prepared from three groups of piglets: Control, ETEC F4 infection (*E. coli*), and ETEC F4 infection with antibiotic supplementation (*E. coli* + ABX). Serum levels of (A) diamine oxidase (DAO) activity, (B) D-lactate, (C) reduced glutathione (GSH), (D) oxidized glutathione disulfide (GSSG), and (F) malondialdehyde (MDA) were

measured. (E) a ratio of GSH to GSSG was calculated based on the values of GSH and GSSG. Results are expressed as mean ± SD (n = 6). $^{*}P < 0.05$ when compared with the control group. $^{\#}P < 0.05$ when compared with the *E. coli* group.

4.4.2 ETEC F4 infection increased oxidative stress in the intestine

A reduction of GSH or a GSH to GSSG ratio as well as an increase in MDA in the serum are an indication of oxidative stress. ETEC F4 infection caused a significant decrease in the GSH level and the GSH to GSSG ratio in the intestine (Figure 4.2 A, B, C). Antibiotic supplementation effectively restored the GSH level and the GSH to GSSG ratio in the intestine of piglets with ETEC F4 infection (Figure 4.2 A, B, C). ETEC F4 infection caused a significant elevation of MDA level in the intestine, indicating increased oxidative stress (Figure 4.2 D). Antibiotic supplementation significantly reduced lipid peroxidation in the intestines of piglets with ETEC F4 infection (Figure 4.2 D). These results suggested that ETEC F4 infection caused oxidative stress in the intestine, while antibiotic supplementation had a protective effect against ETEC F4 infection.

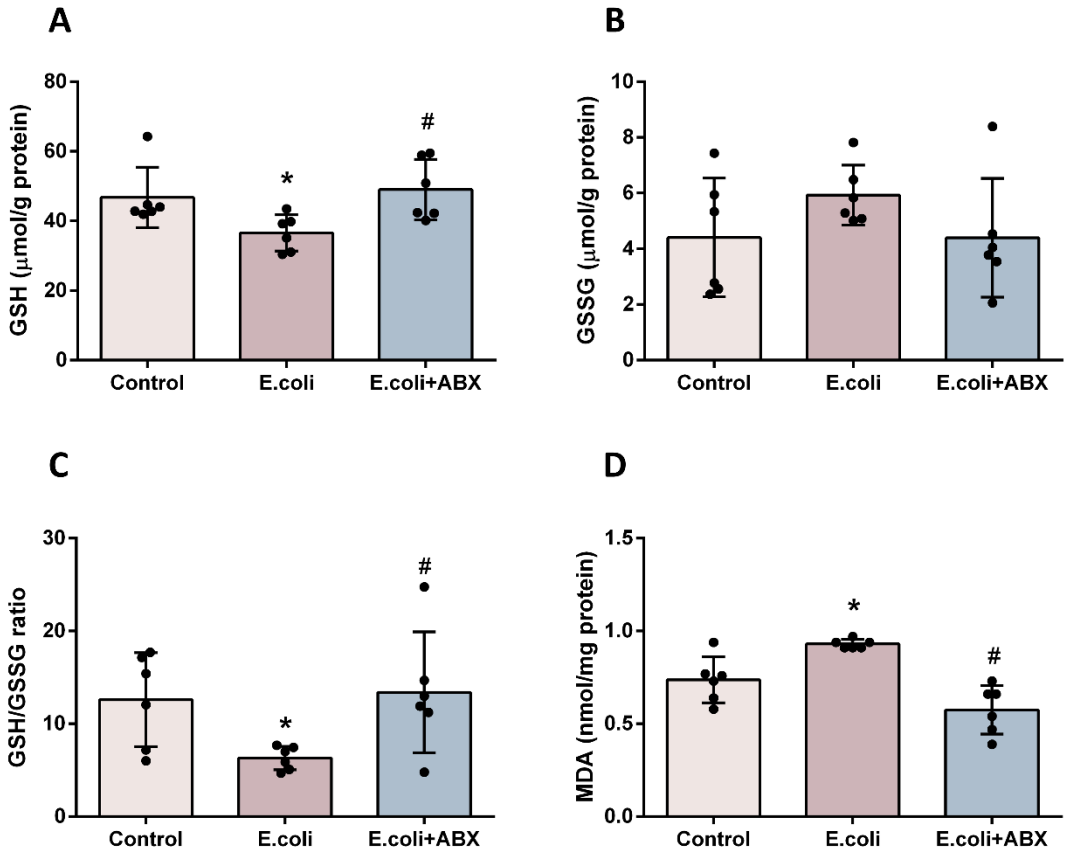

Figure 4.2 Oxidative stress biomarkers in the intestine

Intestinal samples were prepared from the three groups of piglets: Control, ETEC F4 infection (*E. coli*), and ETEC F4 infection with antibiotic supplementation (*E. coli* + ABX). The intestine (A) reduced glutathione (GSH) and (B) oxidized glutathione disulfide (GSSG), were measured. (C) A ratio of GSH to GSSG was calculated based on the values of GSH and GSSG. (D) The intestinal malondialdehyde (MDA) was also measured. Results are expressed as mean ± SD (n = 6). $^{*}P < 0.05$ when compared with the control group. $^{\#}P < 0.05$ when compared with the *E. coli* group.

4.4.3 Effect of ETEC F4 infection on glutathione synthesizing enzymes in the intestine

To examine the effect of infection on glutathione synthesis, we measured the expression of glutathione-synthesizing enzymes in the intestine. ETEC F4 infection significantly reduced the mRNA and protein levels of glutamate-cysteine ligase catalytic subunit (Gclc) and modifier subunit (Gclm), and glutathione synthetase (GS) in the intestine (Figure 4.3). Antibiotic supplementation effectively increased the expression of mRNA and protein of these enzymes in the intestine of piglets with ETEC F4 infection (Figure 4.3).

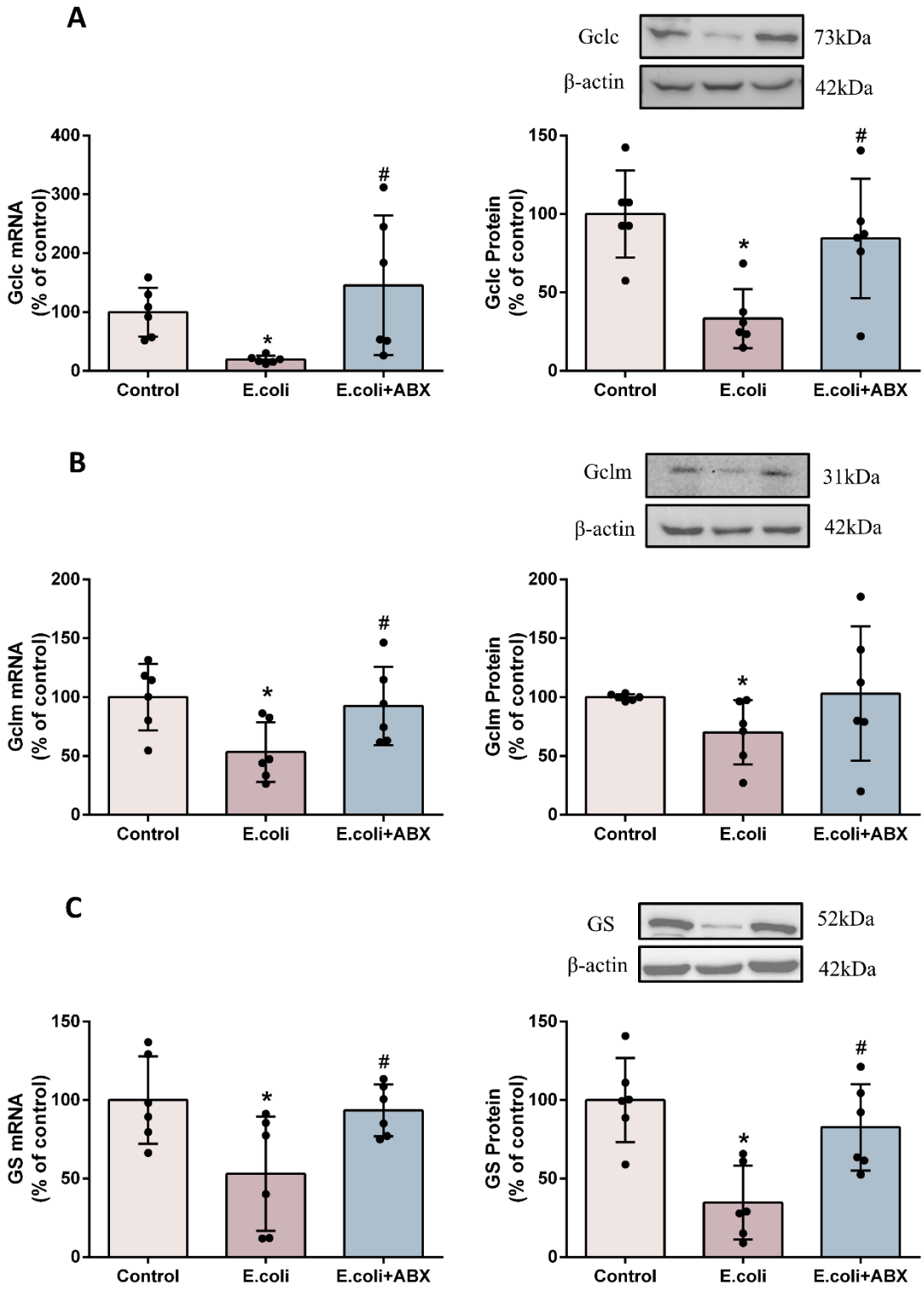

Figure 4.3 Expression of glutathione synthesizing enzymes in the intestine

Intestinal samples were prepared from the three groups of piglets: Control, ETEC F4 infection (*E. coli*), and ETEC F4 infection with antibiotic supplementation (*E. coli* + ABX). The mRNA and protein of (A) glutamate-cysteine ligase catalytic subunit (Gclc), (B) glutamate-cysteine ligase modifier subunit (Gclm), and (C) glutathione synthetase (GS) in the intestine were determined by real-time PCR and Western immunoblotting analysis. Results are expressed as mean ± SD (n = 6). $^{*}P < 0.05$ when compared with the control group. $^{\#}P < 0.05$ when compared with the *E. coli* group.

4.4.4 Effect of ETEC F4 infection on transcription factor Nrf2 and antioxidant enzyme expression in the intestine

To investigate whether antibiotic supplementation affected Nrf2 signaling and antioxidant enzyme expression, we examined the expression of Nrf2, HO-1, and SOD1 in the intestine. ETEC F4 infection decreased the nuclear Nrf2 level in the intestine, indicating the suppression of Nrf2 activation (Figure 4.4 A). Antibiotic supplementation restored the nuclear Nrf2 level in the intestine of piglets with ETEC F4 infection (Figure 4.4 A). Aside from glutathione synthesizing enzymes, the expressions of other key antioxidant enzymes such as SOD1 and HO-1 are also regulated by Nrf2 in response to oxidative stress. ETEC F4 infection reduced HO-1 and SOD1 expression in the intestine, while antibiotic supplementation effectively restored the expression of those antioxidant enzymes (Figure 4.4 B, C). These results suggested that antibiotic supplementation could activate Nrf2 and increase antioxidant enzyme expression in the intestine of piglets with ETEC F4 infection.

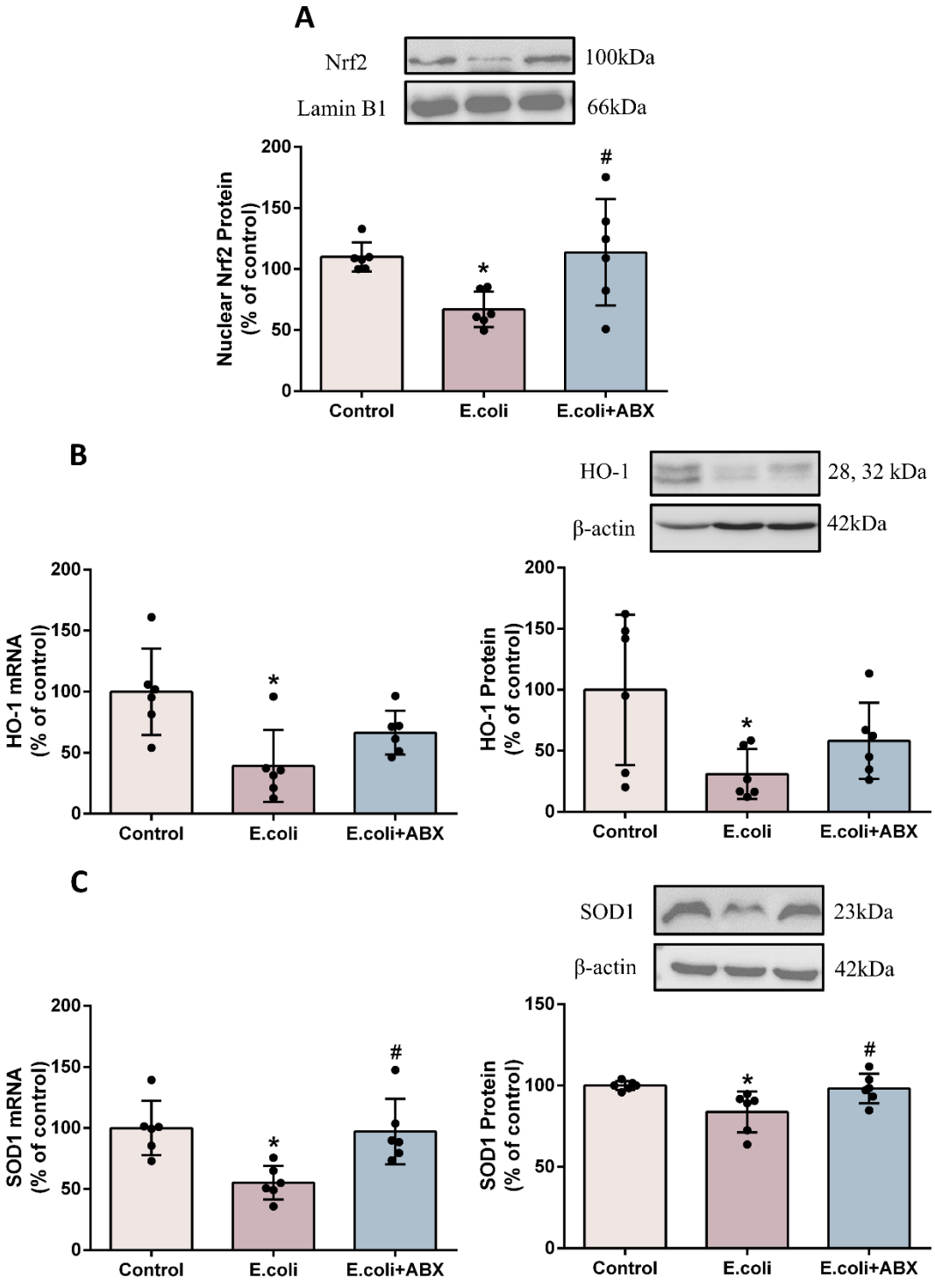

Figure 4.4 Expression of Nrf2, HO-1, and SOD1 in the intestine

Intestinal samples were prepared from the three groups of piglets: Control, ETEC F4 infection (*E. coli*), and ETEC F4 infection with antibiotic supplementation (*E. coli* + ABX). Western immunoblotting analysis measured the (A) nuclear Nrf2 protein expression. The mRNA and protein of (B) heme oxygenase-1 (HO-1) and (C) superoxide dismutase-1 (SOD1) in the intestine were measured by real-time PCR and Western immunoblotting analysis. Results are expressed as mean ± SD (n = 6). $^{*}P < 0.05$ when compared with the control group. $^{\#}P < 0.05$ when compared with the *E. coli* group.

4.4.5 Effect of ETEC F4 infection on tight junction protein and inflammatory cytokines expression in the intestine

The tight junction protein expression in the intestine can be used as a biomarker of intestinal integrity. ETEC F4 infection significantly decreased the expression of tight junction proteins (ZO-1, occludin) in the intestine, while antibiotic supplementation effectively restored tight junction protein expression to the level found in the control group (Figure 4.5). These results suggested that ETEC F4 infection impaired intestinal epithelial barrier integrity, and antibiotic supplementation had a protective effect against ETEC F4 infection (Figure 4.5). ETEC F4 infection also significantly increased the expression of inflammatory cytokines (IL-6, TNF-α, IL-1β), while antibiotic supplementation effectively decreased inflammatory cytokine expression in the intestine (Figure 4.6). ETEC F4 infected group had elevated rectal temperature 24 h after infection (39.52 ± 0.31 vs 39.03 ± 0.26 in control group) and increased diarrhea score 36 h after infection (1.83 ± 0.98 vs 0.03 ± 0.52 in control group).

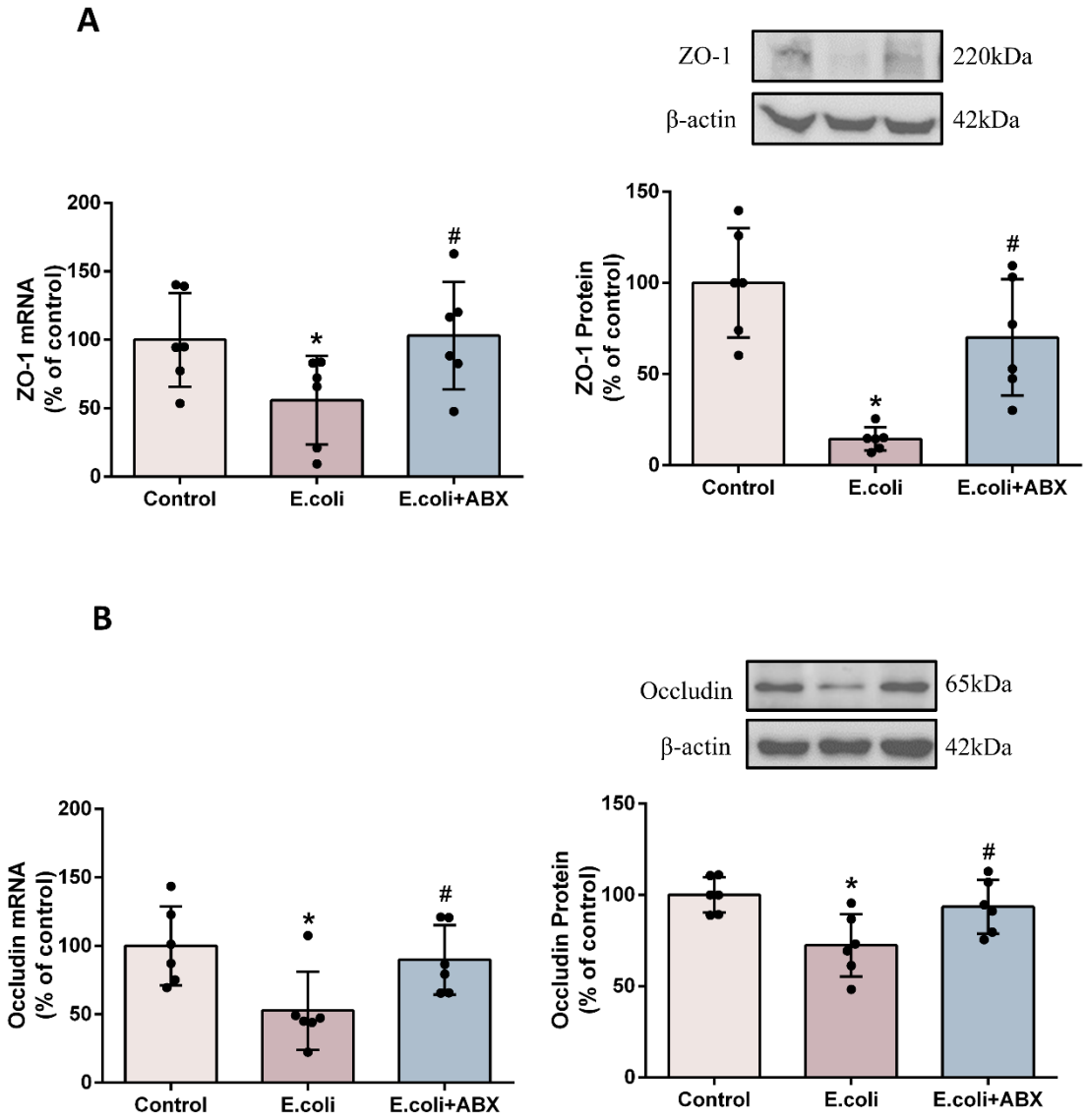

Figure 4.5 Expression of tight junction proteins in the intestine

Intestinal samples were prepared from three groups of piglets: Control, ETEC F4 infection (*E. coli*), and ETEC F4 infection with antibiotic supplementation (*E. coli* + ABX). The mRNA and protein of tight junction proteins (A) Zonula occludens-1 (ZO-1) and (B) occludin in the intestine were measured by real-time PCR and Western immunoblotting analysis. Results are expressed as mean ± SD (n = 6). $^{*}P < 0.05$ when compared with the control group. $^{\#}P < 0.05$ when compared with the *E. coli* group.

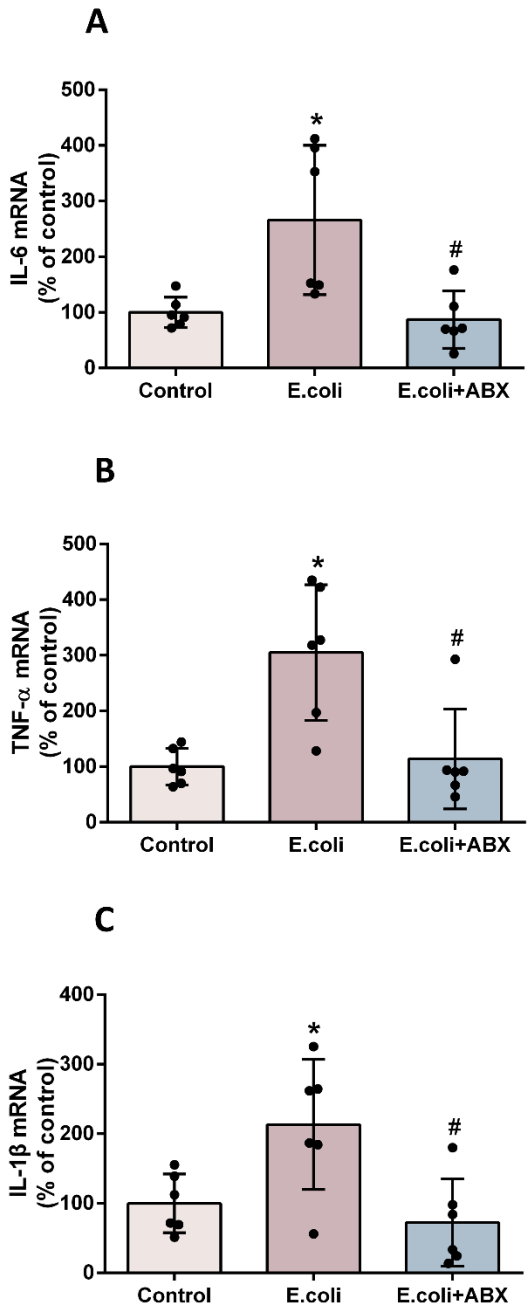

Figure 4.6 Expression of inflammatory cytokines in the intestine

Intestinal samples were prepared from three groups of piglets: Control, ETEC F4 infection (*E. coli*), and ETEC F4 infection with antibiotic supplementation (*E. coli* + ABX). The mRNA of (A) interleukin-6 (IL-6), (B) tumor necrosis factor-α (TNF-α), and (C) Interleukin-1 beta (IL-1β) in the intestine were measured by real-time PCR. Results are

expressed as mean ± SD (n = 6). $^{*}P < 0.05$ when compared with the control group. $^{\#}P < 0.05$ when compared with the *E. coli* group.

4.4.6 Effect of oxidative stress on tight junction protein and inflammatory cytokine expression in enterocytes

To induce oxidative stress, Caco2 cells were incubated with hydrogen peroxide (H_2O_2), which is a widely used agent to induce oxidative stress. Incubation of cells with H_2O_2 (400 or 800 μM) for 4 h resulted in a significant increase in intracellular lipid peroxidation compared to the control (Figure 4.7 A). There was a significant decrease in the level of tight junction protein expression in cells incubated with H_2O_2 (Figure 4.7 B, C). Incubation of cells with H_2O_2 also caused a significant increase in the expression of inflammatory cytokines (IL-6, TNF-α) (Figure 4.7 D, E). The effect of H_2O_2 on cell viability was also examined (Figure 4.7 F). Furthermore, the addition of antioxidant glutathione to the culture medium not only attenuated H_2O_2-induced oxidative stress but also effectively decreased the expression of inflammatory cytokines (IL-6, TNF-α) (Figure 4.8 A, B, C). Such treatment also restored the expression of tight junction proteins (ZO-1 and occludin) in the cells (Figure 4.8 D, E). The H_2O_2 treatment stimulated Nrf2 mRNA expression (Figure 4.8 F), indicating an antioxidant defense response to oxidative stress in the cells.

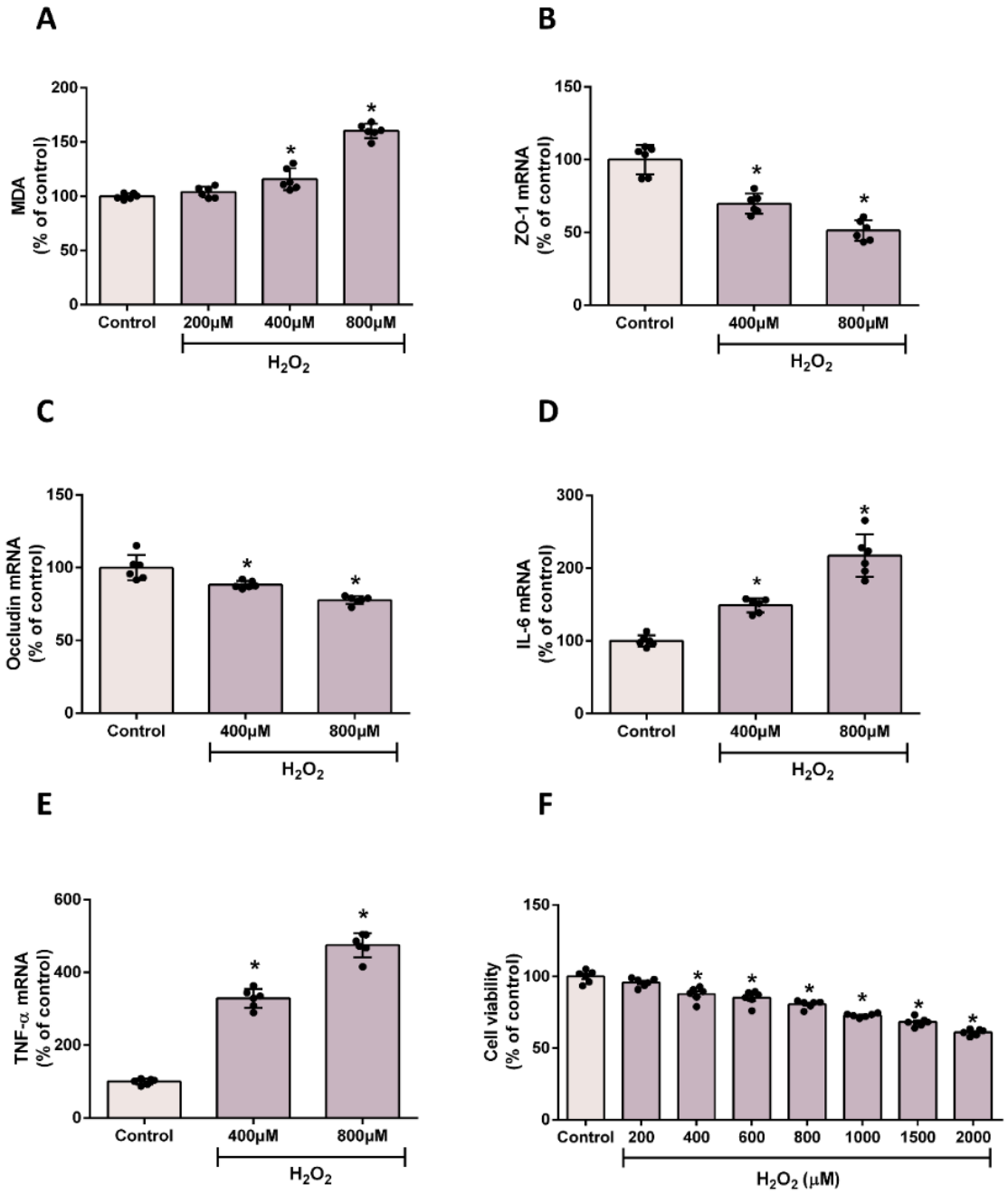

Figure 4.7 Effect of hydrogen peroxide on lipid peroxidation, the expression of tight junction proteins and inflammatory cytokines in Caco2 cells

Cells were incubated in the absence (control) or the presence of hydrogen peroxide (H_2O_2) at 200, 400, or 800 μM for 4 h and (A) Intracellular malondialdehyde (MDA) levels were measured. In another set of experiments, cells were incubated in the absence (control) or

presence of H_2O_2 (400 μM, 800 μM) for 4 h. The mRNA of (B) Zonula occludens-1 (ZO-1), (C) occludin, (D) interleukin-6 (IL-6) and (E) tumor necrosis factor-α (TNF-α) were measured by a real-time PCR analysis. (F) Cell viability was measured after cells were incubated with H_2O_2 (200 to 2000 μM). Results are expressed as mean ± SD (n = 6). $^{*}P <$ 0.05 when compared to the control.

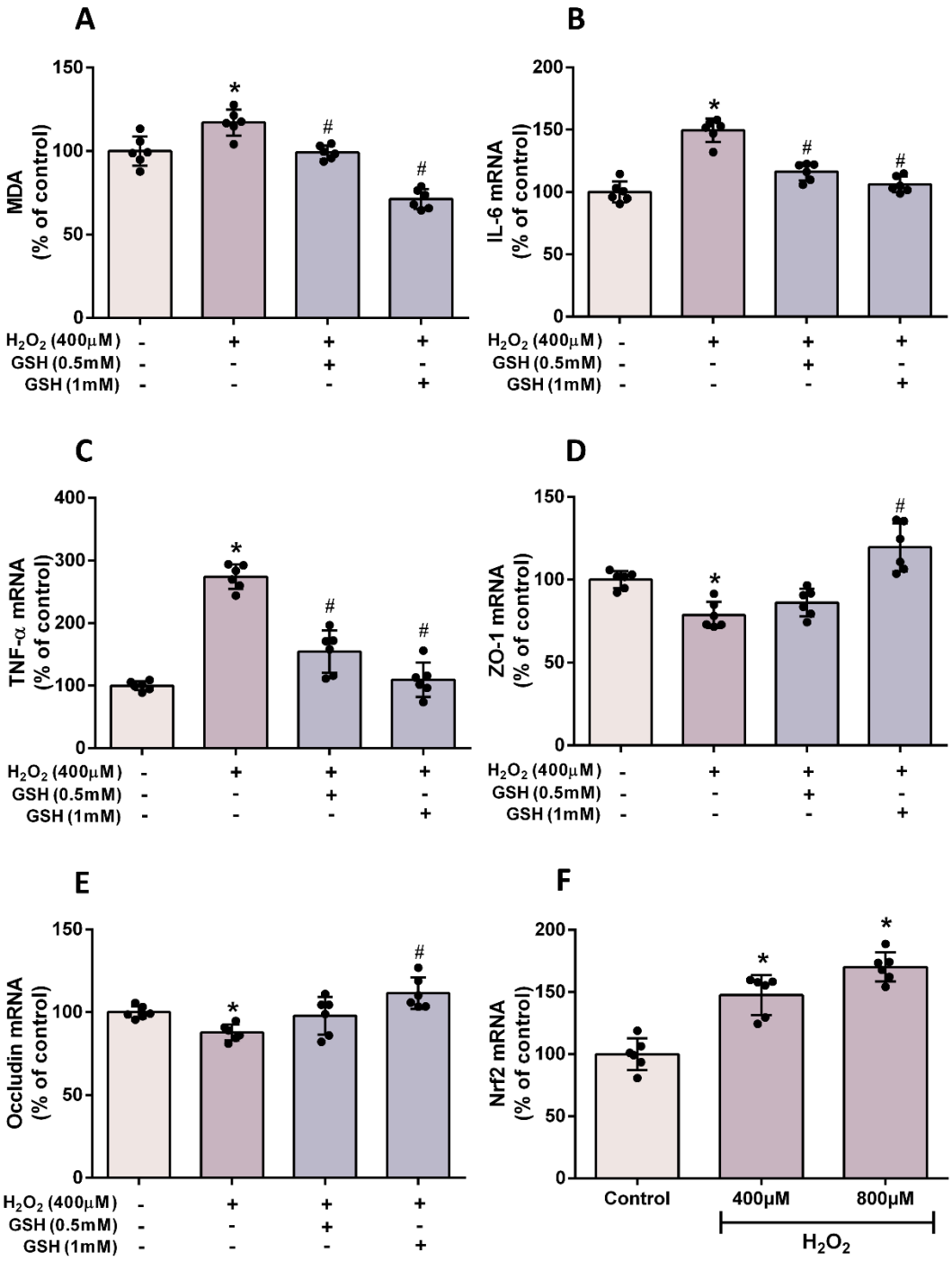

Figure 4.8 Effect of glutathione and hydrogen peroxide on oxidative stress, the expression of tight junction proteins and inflammatory cytokines in Caco2 cells

Cells were incubated with glutathione (GSH) at 0.5 or 1 mM for 30 min, followed by incubation with hydrogen peroxide (H_2O_2 400 μM) for 4 h. (A) Intracellular malondialdehyde (MDA) was measured. The mRNA of (B) interleukin-6 (IL-6), (C) tumor necrosis factor-α (TNF-α), (D) Zonula occludens-1 (ZO-1) and (E) occludin were measured by a real-time PCR analysis. In another set of experiments, cells were incubated in the absence (control) or presence of H_2O_2 (400, 800 μM) for 4 h. The mRNA of (F) Nrf2 was measured by a real-time PCR analysis. Results are expressed as mean ± SD (n = 6). $^{*}P < 0.05$ when compared to the control. $^{\#}P < 0.05$ when compared with the H_2O_2 group.

4.5 Discussion

In the present study, *E. coli* (ETEC F4) infection impaired gut integrity as demonstrated by a significant reduction of tight junction protein expression in the intestine and a marked elevation of DAO activity and D-lactate levels in the serum. The ETEC F4-infected piglets displayed increased oxidative stress biomarkers and pro-inflammatory cytokine expression in the intestine. Downregulation of Nrf2 activation and decreased antioxidant defense contributed to increased oxidative stress in the intestine and in the circulation. Antibiotic supplementation effectively attenuated oxidative stress and improved gut integrity. Furthermore, inhibition of oxidative stress by glutathione increased tight junction protein expression and reduced inflammatory cytokine expression in epithelial Caco2 cells.

Bacterial infection can cause gastrointestinal disorders in humans and animals. ETEC infection is a common cause of diarrhea in travelers and malnutrition in children in developing countries (WHO, 2021; Zhang et al., 2022b; Khalil et al., 2023). In piglets, ETEC is also a well-known pathogen causing post-weaning diarrhea, leading to nutrient malabsorption and growth retardation (Kim et al., 2022b). Understanding the mechanisms of cellular response to infection is crucial to the prevention and mitigation of gastrointestinal disorders. ETEC infection has been shown to cause intestinal dysfunction with increased gut permeability (Kim et al., 2022a). In the present study, piglets with ETEC F4 infection had decreased expression of tight junction proteins (occludin, ZO-1) in the intestine with increased gut permeability. Occludin and ZO-1 are the integral proteins of intestinal epithelial tight junctions. These proteins play a pivotal role in maintaining barrier integrity to ensure selective molecule passage between the gut lumen and the bloodstream.

A decrease in the expression of tight junction proteins suggested that the integrity of the intestine was compromised. DAO is present primarily in the intestinal mucosa, and its elevation in the blood circulation indicates early intestinal barrier damage (Liu et al., 2020c). D-lactate is a metabolic byproduct of intestinal bacteria. Under physiological circumstances, only a minimal amount of D-lactate reaches the bloodstream. However, a compromised intestinal barrier can result in a significant increase in serum D-lactate levels. Taken together, a decrease in tight junction protein expression with an increase in serum DAO activity and D-lactate level indicated that the intestinal barrier function was impaired in the piglets with ETEC F4 infection.

Increased oxidative stress can elicit inflammatory response and impair gut function (Chami et al., 2018). In the present study, ETEC F4 infection caused oxidative stress that was accompanied by an increased expression of inflammatory cytokines in the intestine. Glutathione is an important endogenous antioxidant in mammals (Vairetti et al., 2021). Its depletion correlated with increased oxidative stress in patients with chronic ulcerative colitis (Koch et al., 2000; Halliwell, 2024). In the present study, glutathione synthesis was compromised in the intestine, as evidenced by a decreased expression of glutathione synthesizing enzymes and low GSH level in the piglets with ETEC F4 infection. Nrf2 is a key transcription factor that regulates the expression of genes involved in antioxidant defense. Nrf2 is sensitive to redox status, and its activation enhances antioxidant defenses by upregulating the expression of antioxidant enzymes such as HO-1, glutathione synthetase, and SOD1 (Hybertson et al., 2011). In the present study, ETEC F4 infection decreased the nuclear level of Nrf2, indicating that Nrf2 activation was reduced in the

intestine (Figure 4.9). This was accompanied by a significant reduction of its target gene expression including antioxidant enzymes (HO-1, SOD) and glutathione synthesizing enzymes. These results suggested that altered Nrf2 activation could diminish antioxidant defense, leading to oxidative stress in the intestine of the piglets with ETEC F4 infection (Figure 4.9). Use of antibiotics is an effective strategy to counteract the harmful effects of ETEC infection in humans and piglets. In the present study, antibiotic supplementation restored Nrf2 activation and antioxidant enzyme expression in the intestine. Such treatment also reduced inflammatory cytokine expression and improved the integrity of the intestine. A recent study showed that use of probiotic (LR-LFCA) increased nuclear Nrf2 protein and HO-1 expression in the ileum of *E. coli* challenged piglets (Xie et al., 2021). The results suggested that restoration of Nrf2 signaling pathway could increase antioxidant capacity in piglets after infection. Our results showed that induction of oxidative stress in gut epithelial cell (Caco2) led to the inhibition of tight junction protein expression and an elevation of inflammatory cytokine expression. Antioxidant glutathione not only alleviated oxidative stress but also increased tight junction protein expression and attenuated inflammatory cytokine expression in Caco2 cells. Future studies may include experiments conducted in epithelial cells isolated from piglet intestine to complement the findings obtained from Caco2 cell line. The results obtained from cultured epithelial cells also need to be corroborated with the in vivo findings. Taken together, our results suggested that attenuation of oxidative stress might improve intestinal function.

To the best of our knowledge, this is the first study reporting that altered Nrf2 activation diminished non-enzymatic and enzymatic antioxidant defense, leading to oxidative stress

in the intestine of piglets with ETEC F4 infection. Our in vivo and in vitro results support a notion that oxidative stress may contribute to increased gut permeability and inflammatory response. It was reported that ETEC infection was associated with altered gut microbiota and the immune system (Bin et al., 2018). Supplementation of antioxidants such as polyphenols could influence gut microbiota composition and promote intestinal health (Catalkaya et al., 2020). Although we observed that attenuation of oxidative stress in Caco2 cells could improve tight junction protein expression and reduce inflammatory cytokine expression, it remains to be investigated whether restoration of antioxidant defense can improve gut health.

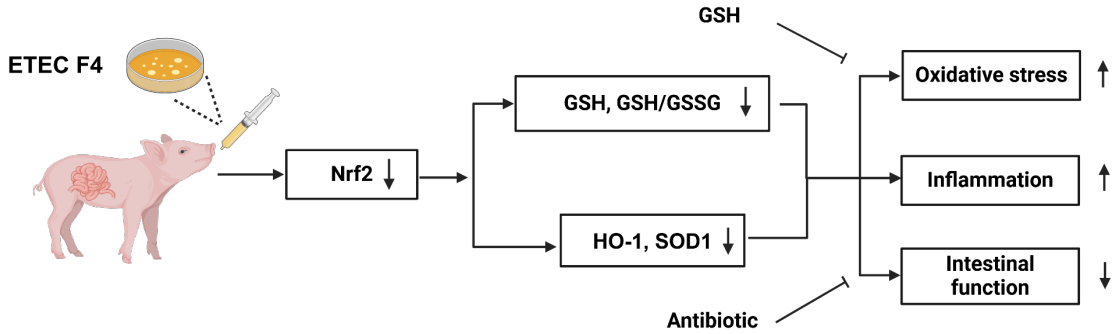

Figure 4.9 Mechanisms of infection induced oxidative stress and intestinal dysfunction

ETEC F4 infection impaired the Nrf2 signaling pathway in piglets, leading to reduced expression of enzymes involved in antioxidant defense (SOD1 and HO-1), and decreased GSH and GSH/GSSG ratio. This downregulation increased oxidative stress, promoted inflammation, and impaired intestinal barrier integrity, leading to intestinal dysfunction. Antibiotic supplementation to piglets or addition of GSH to epithelial cells restored antioxidant defense and intestinal function. Abbreviations: ETEC F4, enterotoxigenic *Escherichia coli* F4; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; Nrf2, nuclear factor erythroid 2-related factor 2; SOD1, superoxide dismutase-1; HO-1, heme oxygenase-1.

4.6 Conclusions

In summary, our study has shown that ETEC F4 infection induces oxidative stress by downregulating Nrf2 signaling and inhibiting glutathione biosynthesis in the intestine. Increased oxidative stress is associated with increased inflammatory response and impaired gut integrity. Antibiotic supplementation can restore glutathione biosynthesis and the expression of antioxidant enzymes in the intestine. Our results suggest that mitigation of oxidative stress is associated with a reduction of inflammatory response and an improvement of intestinal integrity. Regulation of redox status through restoration of antioxidant defense may improve intestinal function.

The World Health Organization estimated that ETEC infection caused about 220 million global episodes of diarrhea. Considering this significant impact of ETEC infections on causing gastrointestinal disorders from incidences such as increased frequency of contamination in packaged salad, outbreaks on cruised ships, traveler's diarrhea and malnutrition in children in developing countries, our findings are vital. They highlight the importance of understanding and potentially managing oxidative stress in the gut not only for animal health but also for crucial food safety and public health issues. This research presents alternative therapeutic (cf. vaccine) strategies and opens avenues for further studies to explore effective ways to control and prevent gastrointestinal disturbances caused by bacterial infections in diverse populations, thereby improving health outcomes in both humans and animals.

Transition statement

Study 2 identified the mechanisms of ETEC infection-induced oxidative stress and its impact on gut function. It became clear that ETEC leads to intestinal oxidative stress and disrupts the key antioxidant pathways. While Study 2 highlighted the important role of oxidative stress and the depletion of glutathione in ETEC-induced intestinal dysfunction, the mechanisms by which the oxidative imbalance could be counteracted remained to be further explored. Study 3 investigated if attenuation of oxidative stress by probiotics could alleviate oxidative stress and improve gut function. Probiotics such as *Bacillus licheniformis* have been recognized for their ability to modulate gut microbiota, enhance antioxidant enzyme activity, and support intestinal barrier integrity (Sun et al., 2023; Yu et al., 2023). Study 3 aimed to determine how *Bacillus licheniformis* HG76 influenced glutathione synthesis, Nrf2 signaling, and antioxidant enzyme expression to counteract ETEC-induced oxidative damage. By transitioning from understanding the pathogenic effects of ETEC to evaluating targeted probiotic interventions, this research provides new insights into the role of oxidative stress in intestinal health management.

CHAPTER FIVE: MANUSCRIPT III

Mitigation of intestinal oxidative stress by *Bacillus licheniformis* HG76 in piglets challenged with enterotoxigenic *Escherichia coli*

Shunshun Jin, Haoxiang Xu, Chengbo Yang, Karmin O

Pending submission to *Journal of Animal Science*

5.1 Abstract

Enterotoxigenic *Escherichia coli* infection compromises intestinal barrier integrity, induces oxidative stress, and triggers inflammatory responses, causing a major threat to intestinal health. Although the glutathione (GSH) synthesis pathway and Nrf2 signaling are critical for maintaining redox homeostasis, the mechanisms through which probiotic regulate GSH synthesis and Nrf2 signaling in the context of *E. coli* infection remain unexplored. This study investigated the protective mechanisms of *Bacillus licheniformis* HG76 in mitigating oxidative stress and intestinal dysfunction caused by *E. coli* infection. A total of 28 piglets were assigned to four groups: a control group, an *E. coli*-infected group, and two *E. coli*-infected groups supplemented with either antibiotic (avilamycin) or probiotic (*Bacillus licheniformis* HG76). *E. coli* infection significantly elevated serum diamine oxidase (DAO) activity and D-lactate levels, indicating compromised intestinal barrier integrity and epithelial damage. The infection also increased malondialdehyde (MDA) levels and altered redox balance by lowering GSH levels and GSH/GSSG ratio in serum and intestine, indicating increased oxidative stress. Probiotic supplementation lowered DAO activity and D-lactate level, reduced MDA level, and restored GSH level and the GSH/GSSG ratio, demonstrating improved intestinal integrity and antioxidant capacity. The probiotic also upregulated GSH-synthesizing enzymes (glutamate-cysteine ligase subunits, glutathione synthetase), which were downregulated due to *E. coli* infection. In addition, the probiotic also enhanced tight-junction protein expression (Zonula occludens-1, occludin) and reduced pro-inflammatory cytokine level, improving intestinal barrier integrity and mitigating inflammation. Furthermore, the probiotic restored Nrf2 signaling suppressed by infection, promoting nuclear translocation and increasing the

downstream antioxidant enzymes (heme oxygenase-1, superoxide dismutase 1) expression. By targeting GSH synthesis and Nrf2 activation, *Bacillus licheniformis* HG76 protects against oxidative damage and maintains gut health during *E. coli* infection, highlighting its therapeutic potential.

Key words: Oxidative stress, Intestine, Probiotic, Piglets, Enterotoxigenic *Escherichia coli*

5.2 Introduction

Enterotoxigenic *Escherichia coli* is the main cause of diarrhea, especially in young animals and humans, which poses a significant health and economic burden on agriculture and public health (Zhou et al., 2021b; Pupa et al., 2022). *E. coli* infection is characterized by the production of enterotoxins that bind to receptors on the epithelial cells of the intestine (Sheikh et al., 2022). This binding causes excessive secretion of chloride and bicarbonate, leading to an influx of water into the intestinal lumen and resulting in diarrhea and dehydration (Zhang et al., 2022b). In addition to direct clinical symptoms, *E. coli* infection can damage the intestinal barrier integrity and elevate oxidative stress in the intestine. Previous studies, including our research, have demonstrated that *E. coli* infection can significantly decrease the expression of tight junction proteins, resulting in increased intestinal permeability (Duan et al., 2022; Jin et al., 2024). Disruption of the intestinal barrier increases the translocation of pathogens and endotoxins into circulation, potentially causing systemic inflammation and multi-organ dysfunction, further impairing animal health. Probiotics have emerged as promising interventions for enhancing intestinal health, with *Bacillus licheniformis* being one of the strains known for its beneficial effects (Kim et al., 2022c). *Bacillus licheniformis* (S6 and PF9) have been shown to enhance gut integrity through upregulation of tight-junction proteins, including Zonula occludens-1 (ZO-1) and occludin, which reduce intestinal permeability during infection (Yun et al., 2022). Additionally, *Bacillus licheniformis* (HJ0135) also influences immune responses by reducing pro-inflammatory cytokine production, helping to maintain gut homeostasis during stress or infection (Yun et al., 2022). Beyond its anti-inflammatory effects, *Bacillus licheniformis* possesses antioxidant properties (Ramirez-Olea et al., 2022; Kumar et al.,

2023). Previous research has indicated that *Bacillus licheniformis* or its exopolysaccharides supplementation can boost antioxidant enzyme activity, lower reactive oxygen species levels, and provide protection against lipid peroxidation (Petrova et al., 2021; Palkovicsné Pézsa et al., 2022). Despite these findings, the mechanisms underlying the protective effects of *Bacillus licheniformis*, particularly its role in antioxidant defenses, remain poorly understood.

Oxidative stress is characterized by an imbalance between the production of ROS and the ability of the antioxidant defense system to neutralize thesis species (Yun et al., 2022). Previous studies have shown that MDA levels, a biomarker of oxidative stress, are increased in the serum and intestinal of piglets infected with *E. coli* (Wu et al., 2021a; Jin et al., 2024). Additionally, our previous study observed significant reductions in antioxidant defense, including decreased antioxidant GSH level and reduced the critical antioxidant enzymes (HO-1, SOD1) expression (Jin et al., 2024). Oxidative stress is known to impair cellular functions, exacerbate inflammation, and further weaken the intestinal epithelial barrier (Mostafavi Abdolmaleky and Zhou, 2024). Oxidative stress can also impair nutrient absorption, disrupt gut microbial composition, and delay recovery from infections, all of which contribute to poor growth performance, increase susceptibility to secondary infections, and reduce overall health in animals. These adverse effects highlight the importance of understanding how targeted interventions can reduce oxidative stress and maintain intestinal redox balance during *E. coli* infection.

Managing oxidative stress is closely related to the synthesis and maintenance of glutathione, which serves as a key intracellular antioxidant defense (Franco and Cidlowski, 2009). GSH, in its reduced form, neutralizes ROS and helps maintain redox homeostasis (He et al., 2017). The production of GSH relies on the activity of essential enzymes. Glutamate-cysteine ligase, composed of a catalytic subunit (Gclc) and a modifier subunit (Gclm), catalyzes the initial and rate-limiting step of GSH synthesis, combining glutamate and cysteine to form γ-glutamylcysteine. This intermediate is subsequently converted to GSH through the action of GS, which adds glycine (Lu, 2013). Deficiencies in these enzymes, as observed during *E. coli* infection, significantly compromise the antioxidant capacity of the intestine, leaving the gut vulnerable to oxidative damage and inflammation (Jin et al., 2024). Nuclear factor erythroid 2-related factor 2 serves as a crucial transcriptional regulator of cellular antioxidant responses (Tonelli et al., 2017). Under normal conditions, Nrf2 is bound in the cytoplasm by Kelch-like ECH-associated protein 1, which facilitates its ubiquitination and subsequent degradation via the proteasome (Kopacz et al., 2020). In the presence of oxidative stress, modifications to Kelch-like ECH-associated protein 1 or shifts in cellular redox status release Nrf2, enabling its translocation to the nucleus (Bellezza et al., 2018). Within the nucleus, Nrf2 interacts with antioxidant response elements in the promoters of its target genes, enhancing the transcription of various antioxidant and detoxifying enzymes (Raghunath et al., 2018). Our previous study has highlighted that *E. coli* infection depletes intracellular GSH levels and disrupts Nrf2 signaling, weakening the intestinal antioxidant defense (Jin et al., 2024). This disruption not only extends oxidative damage but also impairs key repair mechanisms required for intestinal recovery. These findings highlight the importance of developing strategies that

target Nrf2 signaling and GSH synthesis to restore antioxidant defense, restrict inflammation and improve gut health.

However, the role of *Bacillus licheniformis* in modulating the process of GSH synthesis and Nrf2 signaling during *E. coli*-induced oxidative stress remains largely unexplored. Addressing this gap is critical to fully understanding the potential of probiotics in protecting against *E. coli*-induced oxidative damage and preserving intestinal health. This study aims to evaluate the protective role of *Bacillus licheniformis* in mitigating oxidative stress and its influence on the process of GSH synthesis and Nrf2 signaling pathways in the intestine of piglets infected by *E. coli*. We hypothesize that *Bacillus licheniformis* supplementation will enhance antioxidant defenses by upregulating GSH synthesis and activating the Nrf2 pathway, thereby mitigating oxidative stress and preserving intestinal integrity in *E. coli* -infected piglets.

5.3 Materials and methods

5.3.1 Animal trial design

Piglets (TN Tempo × TN70) susceptible to enterotoxigenic *Escherichia coli* F4 infection were identified through MUC4 gene detection based on established protocols (Yang et al., 2016; Sterndale et al., 2019b). The probiotic strain *Bacillus licheniformis* HG76 was provided by Dr. Joshua Gong's laboratory in Guelph. The identity of *Bacillus licheniformis* HG76 was confirmed through 16S rDNA sequencing. *Bacillus licheniformis* HG76 (PRO HG76) was prepared and cultured following previously established protocols (Liu et al., 2020a). Twenty-eight weaned piglets (28 days old) were randomly assigned to four groups:

Group 1 (control), Group 2 (*E. coli* infection), Group 3 (*E. coli* infection + antibiotic, *E. coli* + ABX), and Group 4 (*E. coli* infection + PRO HG76). Each group had seven piglets with similar initial body weights. Groups 1 and 2 received a basal diet based on NRC (2012) guidelines for piglets weighing 6-10 kg. Group 3 was given the same diet with antibiotic avilamycin (800 mg Surmax (10% premix) per kg; Elanco, AB, Canada). Group 4 received the basal diet with *Bacillus licheniformis* HG76 at a concentration of 1.0×10^9 CFU per kg. On day 7, Groups 2, 3, and 4 were orally given *Escherichia coli* F4 (5 mL of 1×10^6 CFU/mL suspension from Veterinary Diagnostic Services Laboratory, MB, Canada) (Choi et al., 2020). Blood samples were collected five days post-infection, processed by centrifugation at 4000 ×g for 10 minutes at 4°C. Mid-jejunum sections were collected and mucosal layers were gently scraped for analysis (Jin et al., 2022). Samples were stored at -80°C. All procedures followed the Canadian Council on Animal Care guidelines and were approved by the University of Manitoba Animal Care Committee (F19-020/2, AC11516).

5.3.2 Biochemical measurements

Diamine oxidase (DAO) activity and D-lactate levels, which reflect intestinal permeability, were determined using commercial assay kits (Cat. nos. ab241004 and ab83429, Abcam Inc., Toronto, ON, Canada). The DAO assay involved the conversion of a substrate to produce hydrogen peroxide (H_2O_2), which was subsequently utilized by an enzyme mix to generate a detectable fluorescent signal. For D-lactate, oxidation by D-lactate dehydrogenase produced a colorimetric change measured at 450 nm after a 30-minute incubation. Both assays were conducted using a microplate reader for detection. Malondialdehyde level, serving as an indicator of oxidative stress, were measured in both

intestinal and serum samples using the Thiobarbituric acid reactive substances assay, following procedures outlined in our previous study (Jin et al., 2022). For glutathione analysis, reduced (GSH) and oxidized (GSSG) forms in intestinal and serum samples were quantified via spectrophotometry, with absorbance measured at 412 nm after appropriate incubation (Jin et al., 2022). Protein concentration in samples is typically measured using the Bradford assay and the resulting color change is quantified spectrophotometrically at 595 nm against a standard curve, providing a basis for normalizing biochemical measurements.

5.3.3 Quantitative polymerase chain reaction (PCR) analysis

Total RNA was extracted from tissue and processed for cDNA synthesis following established protocols (Wijerathne et al., 2022). Quantitative PCR (qPCR) was performed using gene-specific primers and a Bio-Rad real-time PCR system (Mississauga, ON, Canada). β-actin served as the reference gene. Primer sequences were designed with the assistance of the Primer-BLAST tool (Table 5.1).

Table 5.1 Primer sequences used for real-time PCR

Genes	Primer sequences (5'-3')	Accession Number	Size (bp)
Gclc	F: GTCCAGTTGGTCCTGTCTGG R: CGGGAGTCCCTTCGATCATG	XM_021098556.1	127
Gclm	F: TTGGAGCAGCTGTACCAGTG R: GAGCTTCCTGGAAACTCGCT	XM_001926378.4	175
GS	F: GTGCTCAAGCCCCAGAGA R: ATGAGGCTCTCTCCTCACTGTC	NM_001244625.1	119
SOD1	F: GTACCAGTGCAGGTCCTCAC R: TTTGCCAGCAGTCACATTGC	NM_001190422.1	104
HO-1	F: GCTGAGAATGCCGAGTTCAT R: GCTGAGAATGCCGAGTTCAT	NM_001004027.1	142
ZO-1	F: GATCCTGACCCGGTGTCTGA R: TTGGTGGGTTTGGTGGGTTG	XM_021098896.1	200
Occludin	F: GAGAGAGTGGACAGCCCCAT R: TGCTGCTGTAATGAGGCTGC	NM_001163647.2	163
TNF-α	F: TTCCAGCTGGCCCCTTGAGC R: GGCATTGGCATACCCAC	NM_214022.1	143
IL-6	F: AAGGTGATGCCACCTCAGAC R: TCTGCCAGTACCTCCTTGCT	NM_001252429.1	151
β-actin	F: CTGCGGCATCCACGAAACT R: AGGGCCGTGATCTCCTTCTG	NM_001172909.1	147

5.3.4 Western blotting analysis

Tissue protein extracts were prepared using RIPA buffer. Protein quantification was done via the Bio-Rad Protein Assay, and equivalent amounts were separated on SDS-PAGE gels before transfer to PVDF membranes. Membranes were blocked using 5% non-fat milk, followed by overnight incubation at 4°C with primary antibodies (Table 5.2). Detection involved HRP-conjugated secondary antibodies and enhanced chemiluminescence (ECL) for visualization. Protein bands were quantified relative to β-actin as a control.

Table 5.2 Primary antibodies for Western immunoblotting analysis

Protein	Antibody	Dilution factor	Source	Catalog number
Gclc	Rabbit anti-GCLC polyclonal	1:1000	Abcam	ab41463
Gclm	Rabbit anti-GCLM polyclonal	1:1000	Abcam	ab118974
GS	Rabbit anti-GS polyclonal	1:1000	Abcam	ab91591
SOD1	Rabbit anti-SOD1 polyclonal	1:1000	Abcam	ab13498
HO-1	Rabbit anti-HO-1 monoclonal	1:1000	Abcam	ab189491
Nrf2	Rabbit anti-Nrf2 monoclonal	1:1000	Abcam	ab62352
ZO-1	Rabbit anti-ZO-1 polyclonal	1:1000	Invitrogen	61-7300
Occludin	Rabbit anti-Occludin polyclonal	1:1000	Invitrogen	71-1500
β-actin	Rabbit anti-β-actin monoclonal	1:1000	Cell signaling	4970s
Lamin B1	Rabbit anti-Lamin B1 polyclonal	1:1000	Abcam	ab16048

5.3.5 Statistical analysis

Data are presented as means ± standard deviation (SD). Prior to analysis, normality and variances homogeneity were assessed. For datasets with normal distribution, one-way ANOVA was performed, with Welch's correction applied for unequal variances. Non-normally distributed data were analyzed using the Kruskal-Wallis test followed by Dunn's post hoc test for pairwise comparisons. Statistical significance was set at $P < 0.05$. All analyses were conducted using GraphPad Prism software (San Diego, CA, USA).

5.4 Results

5.4.1 *Bacillus licheniformis* HG76 improved intestinal integrity and decreased oxidative stress in the serum

Diamine oxidase (DAO) is an enzyme marker for intestinal mucosal damage, while D-lactate serves as an indicator of intestinal permeability. In this study, *E. coli* infection caused a marked elevation of serum DAO activity and D-lactate levels compared to the control (CON) group (Figure 5.1 A, B), indicating compromised intestinal integrity. Supplementation with either antibiotic (avilamycin [ABX] or the probiotic [*Bacillus licheniformis* HG76, PRO HG76]) significantly lowered serum DAO activity and D-lactate levels, indicating improved intestinal barrier function (Figure 5.1 A, B). Additionally, *E. coli* infection decreased serum GSH level and the GSH/GSSG ratio, suggesting increased oxidative stress and a weakened antioxidant defense, though no significant changes were observed in oxidized glutathione (GSSG) level (Figure 5.1 C-E). Moreover, serum MDA level, a marker of lipid peroxidation, was elevated following infection (Figure 5.1 F). Supplementation with antibiotic or probiotic restored the GSH level, the GSH/GSSG ratio

and the reduced MDA levels, mitigating oxidative stress biomarkers. These results suggested that antibiotic or probiotic supplementation can alleviate *E. coli*-induced intestinal permeability and oxidative stress.

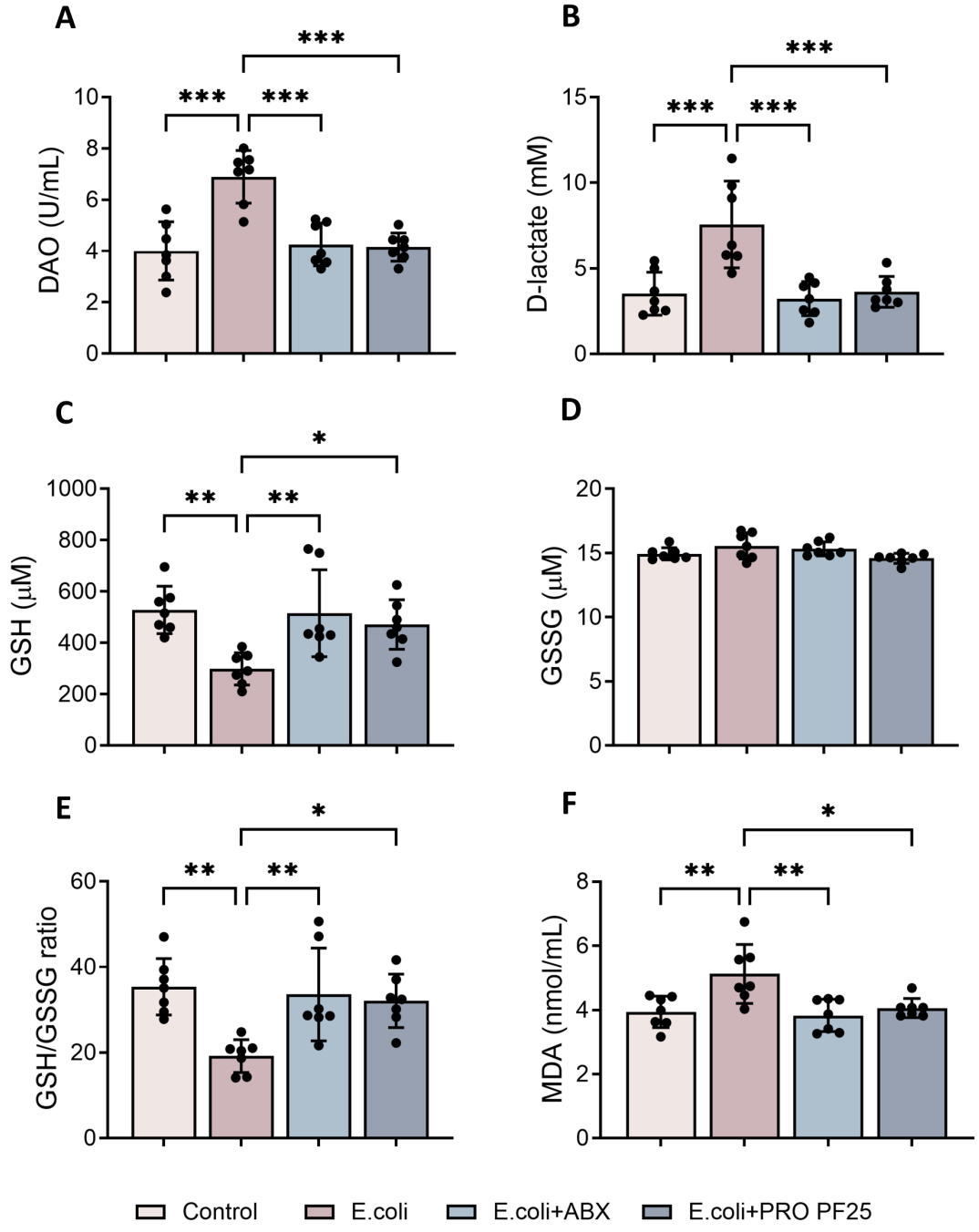

Figure 5.1 Effects of *Escherichia coli* infection and *Bacillus licheniformis* HG76 supplementation on markers of intestinal permeability and oxidative stress

(A) Serum DAO activity, a marker of intestinal permeability and mucosal damage. (B) Serum D-lactate levels, indicating intestinal barrier function. (C) Serum levels of reduced

glutathione (GSH). (D) Serum levels of oxidized glutathione (GSSG). (E) GSH/GSSG ratio as an indicator of redox status. (F) Malondialdehyde (MDA) levels in serum, representing lipid peroxidation and oxidative stress. Data are expressed as mean ± SD (n = 7 per group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Groups included Control, *E. coli*-infected (*E. coli*), *E. coli*-infected with avilamycin (*E. coli* + ABX), *E. coli*-infected with *Bacillus licheniformis* HG76 (*E. coli* + PRO HG76).

5.4.2 *Bacillus licheniformis* HG76 reduced intestinal oxidative stress

The markers such as GSH, the GSH/GSSG ratio, and MDA are commonly used to assess intestinal oxidative stress and redox balance. The reductions in intestinal GSH or the GSH/GSSG ratio and increases in MDA level indicate oxidative stress damage and impaired antioxidant capacity. *E. coli* infection markedly decreased the intestinal GSH level and GSH to GSSG ratio, indicating disrupted antioxidant capacity and increased intestinal oxidative stress (Figure 5.2 A-C). Additionally, *E. coli* infection significantly elevated MDA levels, indicating increased oxidative stress in the intestine (Figure 5.2 D). Supplementation with either antibiotic or probiotic effectively increased the GSH level and the GSH/GSSG ratio and decreased GSSG level, demonstrating their roles in alleviating intestinal oxidative stress (Figure 5.2 A-C). Both antibiotic and probiotic treatments significantly reduced MDA levels, mitigating intestinal oxidative stress damage (Figure 5.2 D). These results indicated that *E. coli* infection induced intestinal oxidative stress, while both antibiotic and probiotic provided protective antioxidative effects, reducing oxidative stress.

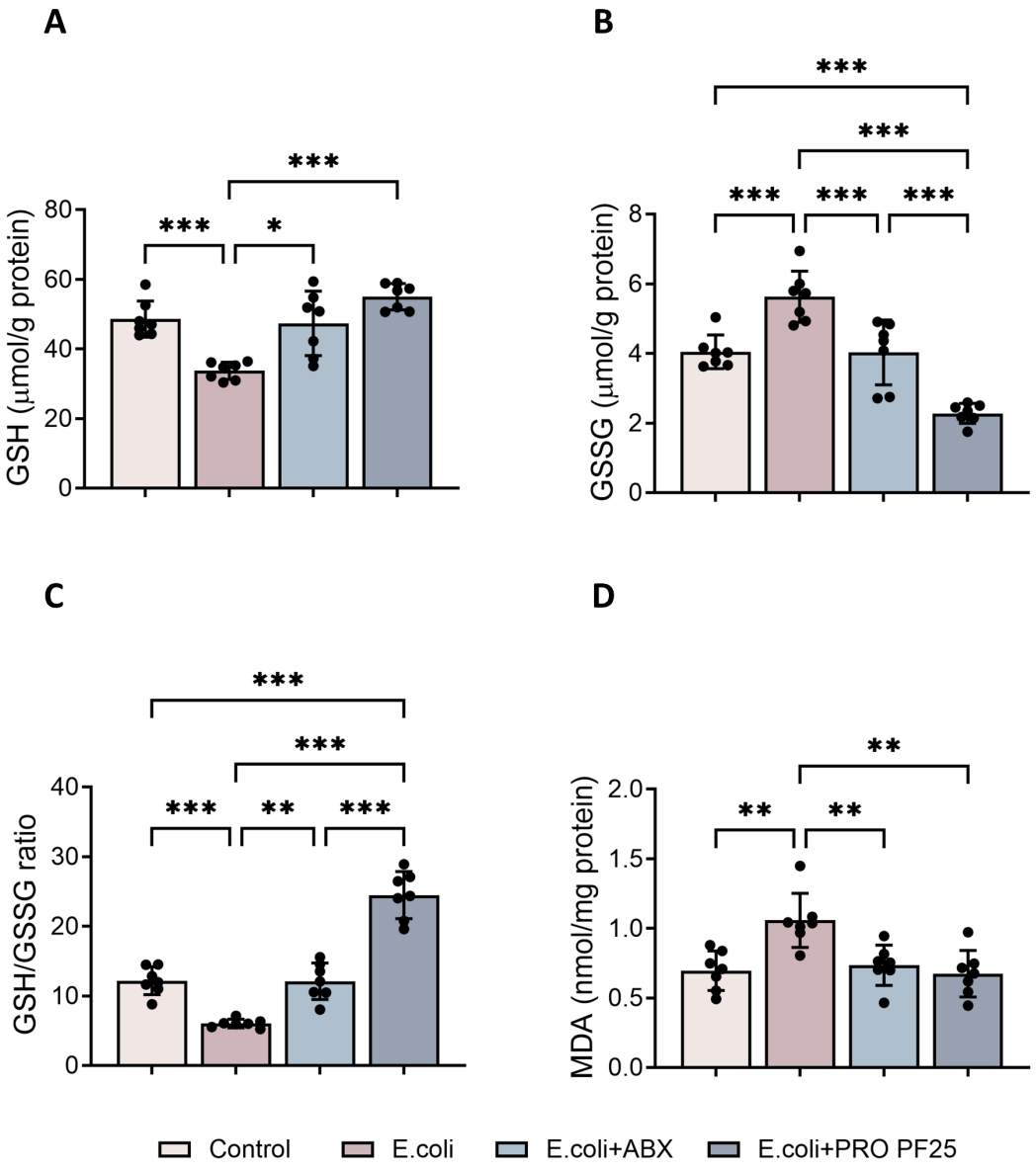

Figure 5.2 Effects of *Escherichia coli* infection and *Bacillus licheniformis* HG76 supplementation on oxidative stress markers in the intestine

(A) Intestinal levels of reduced glutathione (GSH). (B) Intestinal oxidized glutathione (GSSG) levels. (C) GSH/GSSG ratio, indicating redox balance in the intestine. (D) Malondialdehyde (MDA) levels, reflecting lipid peroxidation and oxidative stress. Data are expressed as mean ± SD (n = 7 per group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Groups

included Control, *E. coli*-infected (*E. coli*), *E. coli*-infected with avilamycin (*E. coli* + ABX), *E. coli*-infected with *Bacillus licheniformis* HG76 (*E. coli* + PRO HG76).

5.4.3 Effect of *Bacillus licheniformis* HG76 on glutathione-synthesizing enzymes in the intestine

To explore the mechanisms by which antibiotic and probiotic enhance intestinal GSH levels, we evaluated glutathione-synthesizing enzymes (Gclc, Gclm, GS) expression. *E. coli* infection significantly decreased the mRNA and protein levels of the Gclc, Gclm and GS (Figure 5.3), indicating impaired glutathione synthesis. Supplementation with either antibiotic or probiotic effectively restored the expression of Gclc and GS, demonstrating their protective role in maintaining glutathione synthesis. While Gclm mRNA levels were restored, its protein expression did not return to normal levels following antibiotic or probiotic supplementation. These results indicated that probiotics could partially reverse the inhibitory effect of *E. coli* infection on the glutathione synthesis pathway.

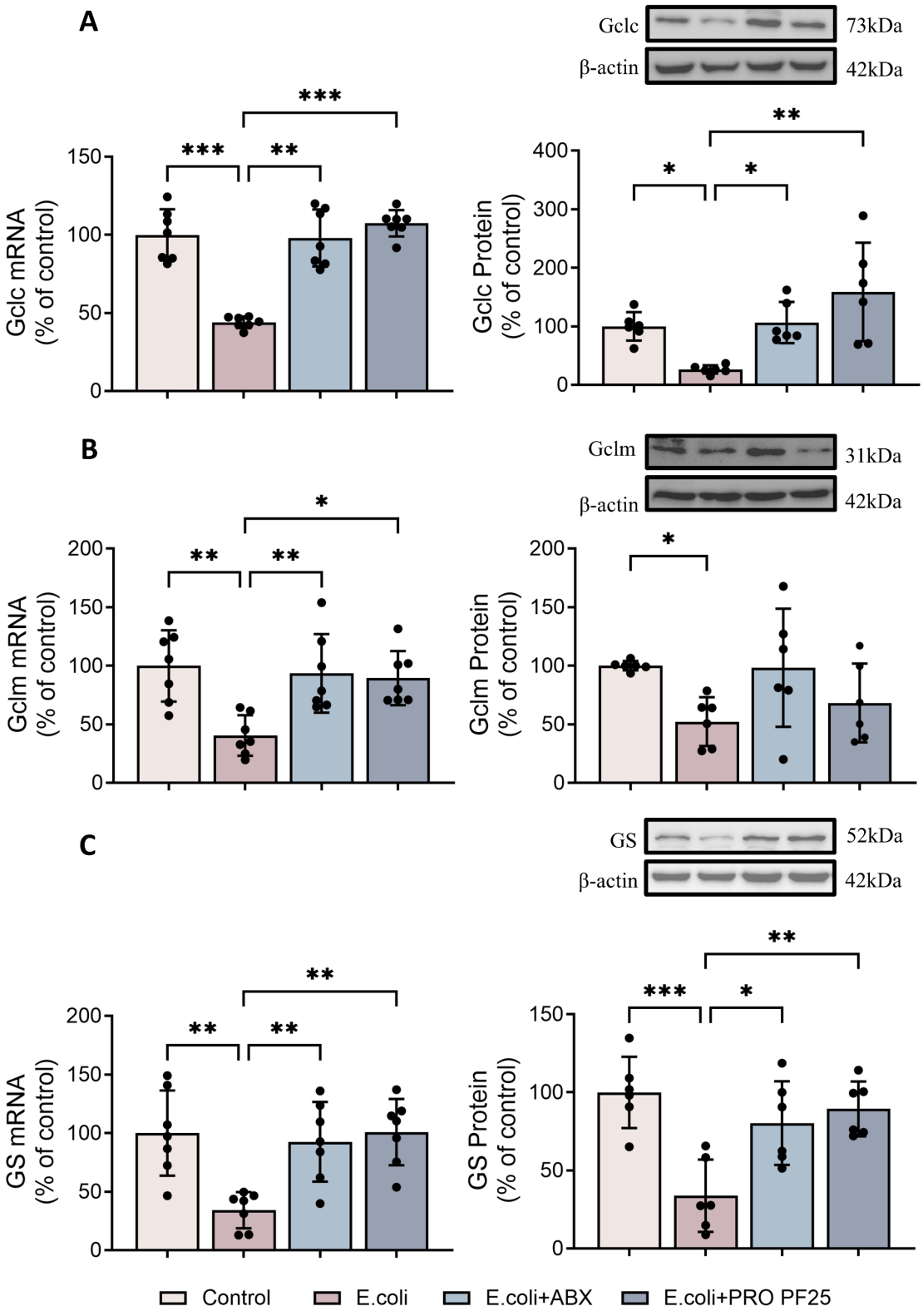
A
Gclc
73kDa
β-actin
42kDa
Gclc mRNA (% of control)
Gclc Protein (% of control)
B
Gclm
31kDa
β-actin
42kDa
Gclm mRNA (% of control)
Gclm Protein (% of control)
C
GS
52kDa
β-actin
42kDa
GS mRNA (% of control)
GS Protein (% of control)
Control
E.coli
E.coli+ABX
E.coli+PRO PF25

Figure 5.3 Effects of *Escherichia coli* infection and *Bacillus licheniformis* HG76 supplementation on expression of GSH-synthesizing enzymes in the intestine

(A) mRNA and protein levels of glutamate-cysteine ligase catalytic subunit (Gclc). (B) mRNA and protein levels of glutamate-cysteine ligase modifier subunit (Gclm). (C) mRNA and protein expression of glutathione synthetase (GS). Gene expression was quantified using real-time PCR, and protein levels were assessed by Western blotting. Data are shown as mean ± SD (n = 6-7 per group). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Groups included Control, *E. coli*-infected (*E. coli*), *E. coli*-infected with avilamycin (*E. coli* + ABX), *E. coli*-infected with *Bacillus licheniformis* HG76 (*E. coli* + PRO HG76).

5.4.4 Effect of *Bacillus licheniformis* HG76 on Nrf2 pathway in the intestine

Nrf2 can regulate downstream antioxidant enzyme expression, such as heme oxygenase-1 (HO-1) and superoxide dismutase 1 (SOD1). To evaluate the role of probiotic in regulating Nrf2 signaling, we analyzed intestinal levels of Nrf2, HO-1 and SOD1. *E. coli* infection suppressed Nrf2 activation by reducing its nuclear level in the intestine (Figure 5.4 A). *E. coli* infection decreased the expression of HO-1 and SOD1, indicating impaired antioxidant defense and increased susceptibility to intestinal oxidative stress. (Figure 5.4 B-C). Supplementation with either antibiotic or probiotic counteracted this effect, restoring Nrf2 nuclear level and supporting its activation. Probiotic supplementation also restored the expression of HO-1 and SOD1, suggesting their capacity to enhance Nrf2 signaling and maintain the intestinal redox balance in the presence of *E. coli*-induced stress.

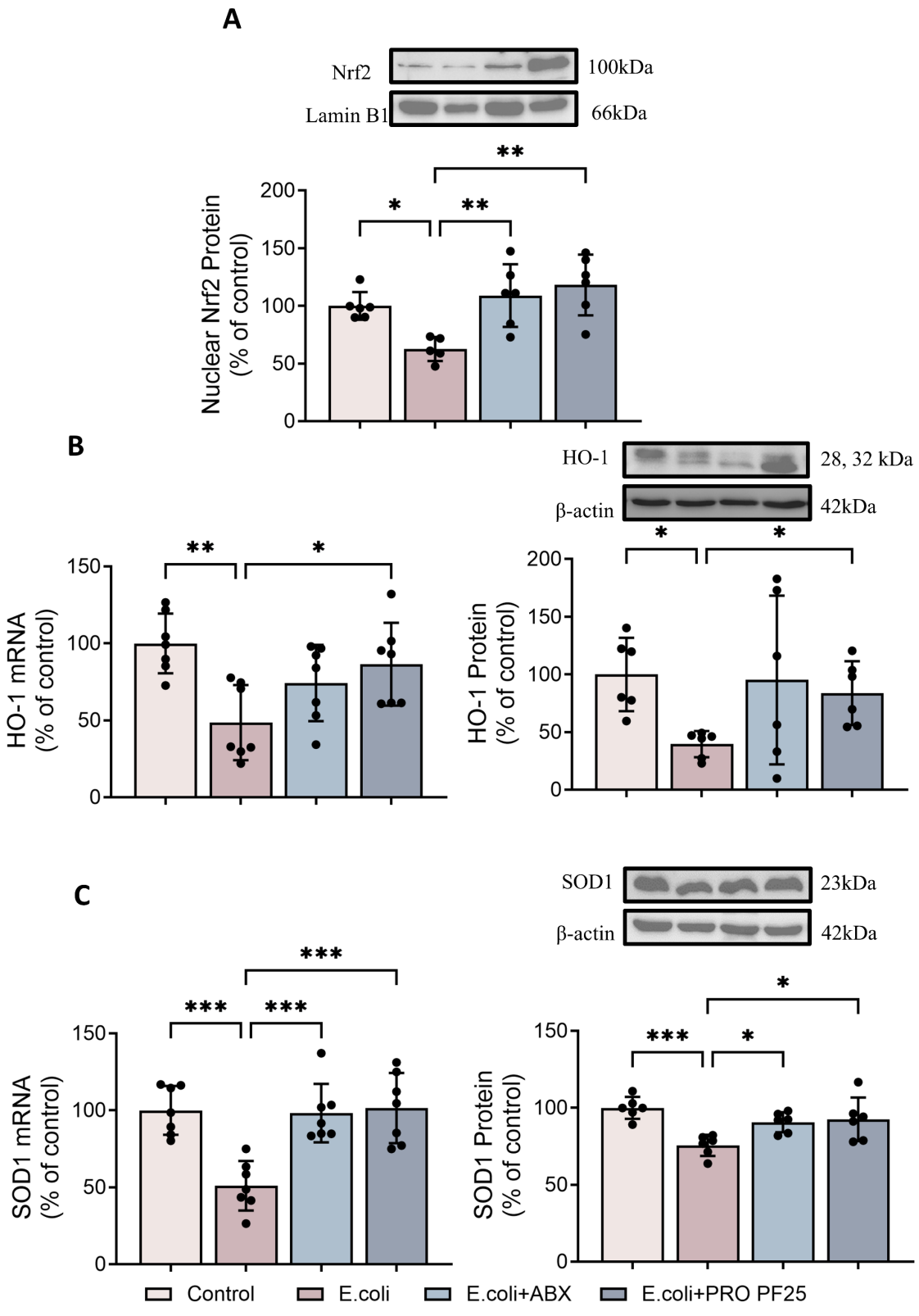
A
Nrf2
100kDa
Lamin B1
66kDa
Nuclear Nrf2 Protein (% of control)
B
HO-1
28, 32 kDa
β-actin
42kDa
HO-1 mRNA (% of control)
HO-1 Protein (% of control)
C
SOD1
23kDa
β-actin
42kDa
SOD1 mRNA (% of control)
SOD1 Protein (% of control)
Control
E.coli
E.coli+ABX
E.coli+PRO PF25

Figure 5.4 Effects of *Escherichia coli* infection and *Bacillus licheniformis* HG76 supplementation on Nrf2 signaling and the expression of antioxidant enzymes in the intestine

(A) Nuclear Nrf2 protein expression, representing Nrf2 activation and translocation. (B) mRNA and protein expression of heme oxygenase-1 (HO-1). (C) mRNA and protein expression of superoxide dismutase 1 (SOD1). Gene expression was determined using real-time PCR, while protein levels were evaluated using Western blotting. Data are presented as mean ± SD (n = 5–7 per group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Groups included Control, *E. coli*-infected (*E. coli*), *E. coli*-infected with avilamycin (*E. coli* + ABX), *E. coli*-infected with *Bacillus licheniformis* HG76 (*E. coli* + PRO HG76).

5.4.5 Effect of *Bacillus licheniformis* HG76 on intestinal tight junction protein expression

Tight-junction proteins, including ZO-1 and occludin, are critical for maintaining intestinal epithelial integrity. Their expression serves as a key indicator of intestinal permeability. Infection with *E. coli* led to a marked reduction in the expression of these proteins, indicating an increased intestinal permeability (Figure 5.5). Supplementation with either antibiotic or probiotic effectively restored the expression of tight-junction proteins, thereby preserving the integrity of the intestinal epithelial barrier. These findings demonstrated that both treatments could protect the intestine from damage caused by *E. coli* infection, thereby enhancing intestinal integrity.

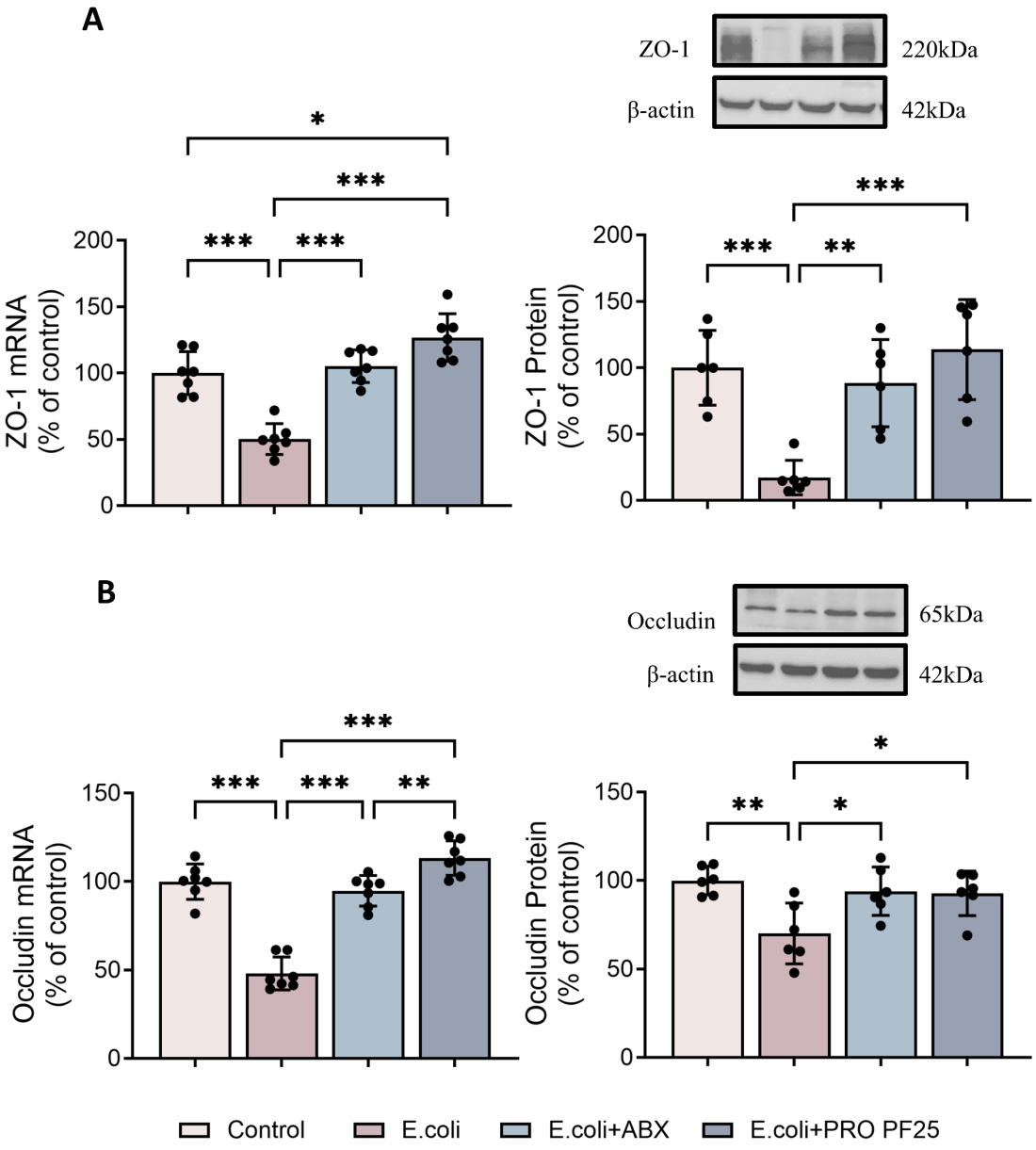

Figure 5.5 Effects of *Escherichia coli* infection and *Bacillus licheniformis* HG76 supplementation on the expression of tight-junction proteins in the intestine

(A) Expression of mRNA and protein levels of zonula occludens-1 (ZO-1). (B) Expression of mRNA and protein levels of occludin. Real-time PCR was used for gene expression quantification, and Western blotting assessed protein levels. Data are shown as mean ± SD (n = 6-7 per group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Groups included Control, *E.*

coli-infected (*E. coli*), *E. coli*-infected with avilamycin (*E. coli* + ABX), *E. coli*-infected with *Bacillus licheniformis* HG76 (*E. coli* + PRO HG76).

5.4.6 Effect of *Bacillus licheniformis* HG76 on inflammatory cytokines in the intestine

Pro-inflammatory cytokines play a critical role in initiating and amplifying the inflammatory response. *E. coli* infection significantly elevated the mRNA levels of proinflammatory cytokines (IL-6, TNF-α, IL-1β), indicating an elevated inflammatory response in the intestine (Figure 5.6 A-C). Supplementation with either antibiotic or probiotics effectively decreased these cytokines mRNA expression, thereby alleviating the inflammatory state caused by *E. coli* infection. These results suggested that probiotic exhibited anti-inflammatory properties, contributing to the regulation of cytokine expression and the attenuation of intestinal inflammation caused by *E. coli* infection.

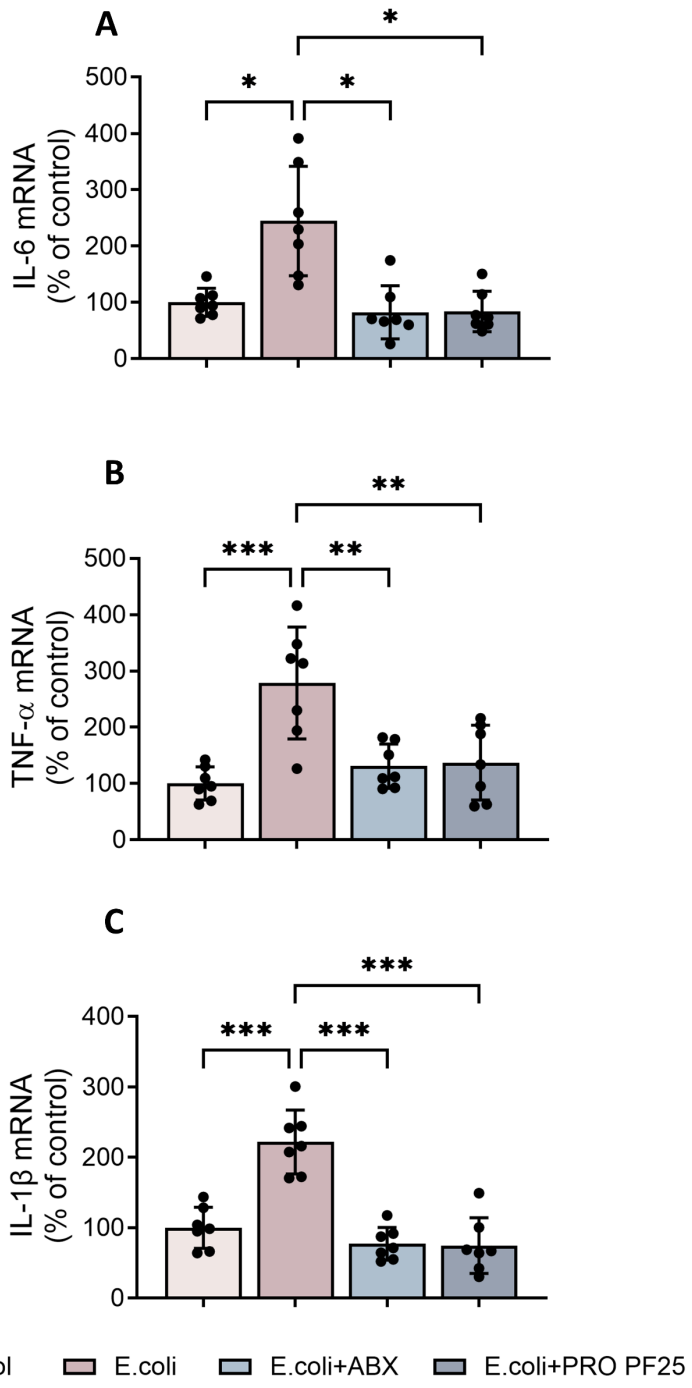

Figure 5.6 Effects of *Escherichia coli* infection and *Bacillus licheniformis* HG76 supplementation on mRNA expression levels of pro-inflammatory cytokines in the intestine

(A) Interleukin-6 (IL-6), (B) Tumor necrosis factor-alpha (TNF-α), and (C) Interleukin-1 beta (IL-1β) mRNA expression was quantified using real-time PCR. Data are expressed as mean ± SD (n = 7 per group). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Groups included Control, *E. coli*-infected (*E. coli*), *E. coli*-infected with avilamycin (*E. coli* + ABX), *E. coli*-infected with *Bacillus licheniformis* HG76 (*E. coli* + PRO HG76).

5.5 Discussion

This study showed that *Bacillus licheniformis* HG76 had a protective effect against oxidative stress and intestinal dysfunction caused by *E. coli* infection, mainly by activating glutathione synthesis and the Nrf2 signaling pathway. In addition to its effects on redox balance, *Bacillus licheniformis* HG76 improved intestinal barrier integrity by enhancing the expression of tight-junction proteins (ZO-1 and occludin) that were disrupted by *E. coli* infection. Furthermore, the probiotic reduced levels of pro-inflammatory cytokines, mitigating inflammation in the intestine. These combined effects highlight the multiple protective roles of *Bacillus licheniformis* HG76 and provide new insights into the antioxidative and anti-inflammatory mechanisms mediated by probiotics, highlighting their therapeutic potential in managing *E. coli*-induced intestinal dysfunction.

E. coli infection increased plasma DAO activity and D-lactate levels, markers of compromised intestinal barrier function (Honzawa et al., 2011; Guo et al., 2019). DAO is an enzyme released from the damaged intestinal mucosa, while D-lactate serves as a marker of intestinal permeability; elevated levels of these markers indicate increased intestinal permeability and damage (Luk et al., 1980; Nielsen et al., 2012). Our results showed that *Bacillus licheniformis* HG76 supplementation significantly lowered DAO activity and D-lactate levels, suggesting a protective role in maintaining intestinal barrier integrity and reducing epithelial damage caused by *E. coli* infection. This aligns with the findings from studies on other probiotic strains, such as *Lactobacillus paracasei* that significantly reduced DAO activity in *E. coli*-challenged mice, improving gut barrier integrity (Ren et al., 2022). Another study showed that *Lactobacillus rhamnosus* LB1 had

a protective effect in *E. coli*-infected piglets by lowering D-lactate levels and restoring tight-junction integrity, highlighting the potential of probiotics in mitigating intestinal permeability and inflammation associated with pathogenic infection (Wu et al., 2021a). Furthermore, probiotic supplementation improved intestinal barrier integrity by restoring the expression of ZO-1 and occludin that were disrupted by *E. coli* infection. Similar results were reported by a previous study where probiotic *Bacillus licheniformis* improved tight-junction integrity and reduced permeability during pathogen-induced gut damage (Xu et al., 2024). The probiotic also exhibited anti-inflammatory properties by reducing pro-inflammatory cytokine expression, thereby mitigating the inflammatory response associated with *E. coli* -induced gut damage. Several studies have confirmed that *Bacillus licheniformis* strains can modulate immune responses, reducing the expression of pro-inflammatory cytokines, and enhancing anti-inflammatory pathways (Yun et al., 2022). This highlights their broad-spectrum anti-inflammatory capabilities in gut health. These combined effects highlight the multiple protective effects of *Bacillus licheniformis* HG76 in maintaining intestinal health during pathogen challenge.

E. coli infection is well-known for disrupting the redox balance in the intestine, leading to increased levels of oxidative stress markers, such as MDA that is a byproduct of lipid peroxidation and a reliable indicator of oxidative damage (Valenzuela, 1991; Wu et al., 2021a; Luo et al., 2022a). Our study demonstrated that supplementation with *Bacillus licheniformis* HG76 significantly reduced the MDA levels in both serum and intestine, highlighting its potent antioxidative properties. This reduction in oxidative stress suggested that *Bacillus licheniformis* HG76 played a protective role in mitigating lipid peroxidation

and restoring redox balance in the gut environment following *E. coli* infection. Oxidative stress is also characterized by changes in glutathione (GSH) levels and the GSH/GSSG ratio (Degroote et al., 2020). GSH, a major intracellular antioxidant, plays a crucial role in neutralizing ROS and maintaining cellular redox homeostasis (He et al., 2017). The GSH/GSSG ratio serves as a key indicator of cellular oxidative stress, with a lower ratio reflecting a compromised antioxidant capacity (Marí et al., 2009). *E. coli* infection leads to a significant reduction in GSH level and a decreased GSH/GSSG ratio in both serum and intestinal tissues, indicating impaired antioxidant defense. We also observed that *Bacillus licheniformis* HG76 supplementation not only restored GSH levels but also increased the expression of key enzymes involved in GSH synthesis, including Gclc, Gclm and GS. This finding provides novel insights, as previous research on probiotics has largely overlooked their role in modulating GSH synthesis via the specific upregulation of these rate-limiting enzymes. By targeting these critical steps in GSH production, *Bacillus licheniformis* HG76 enhances antioxidant defense mechanisms and counteracts *E. coli*-induced oxidative stress, thereby maintaining redox homeostasis. This unique mechanism highlights the probiotic's therapeutic potential and sets it apart as a promising intervention for oxidative stress-related intestinal dysfunction.

The Nrf2 pathway plays a pivotal role in cellular antioxidant defense, regulating the expression of numerous antioxidant enzymes, including HO-1 and SOD1 (He et al., 2017). *E. coli* infection significantly suppressed Nrf2 activation, as indicated by reduced nuclear level of Nrf2. Probiotic supplementation restored Nrf2 activation and increased the expression of downstream antioxidant enzymes. Studies have indicated that probiotic

Clostridium butyricum strains can increase the mRNA expression of Nrf2, leading to increased antioxidant enzyme mRNA expression (HO-1 and SOD1) and reduced oxidative stress markers (Li et al., 2021a). Similarly, *Lactobacillus rhamnosus* LB1 supplementation in piglets has been shown to enhance Nrf2-related mRNA expression, mitigating *E. coli*-induced oxidative stress through its antioxidative and anti-inflammatory actions (Wu et al., 2022). Additionally, *Lactobacillus delbrueckii* subsp. *bulgaricus* exopolysaccharides increased nuclear Nrf2 translocation and elevated mRNA levels of antioxidant enzymes such as SOD, catalase, and HO-1, effectively countering oxidative stress in *vitro* (Liu et al., 2024a). However, although these studies focused on the regulation of Nrf2-related mRNA expression, there is limited evidence of direct regulation of Nrf2 protein activation and its nuclear transport in *E. coli* infection models. Our study demonstrates that *Bacillus licheniformis* HG76 can significantly promote the activation and nuclear transport of Nrf2 protein in *E. coli*-infected piglets, thus providing a more comprehensive explanation of the Nrf2-mediated antioxidant defense mechanism (Figure 5.7). This study highlights potent protective effects of *Bacillus licheniformis* HG76 in mitigating oxidative stress and intestinal dysfunction induced by *E. coli* infection, demonstrating its potential as a therapeutic intervention. By modulating Nrf2 signaling, *Bacillus licheniformis* HG76 enhances intestinal antioxidant capacity and effectively reduces oxidative damage.

To the best of our knowledge, this study provides new evidence that *Bacillus licheniformis* HG76 supplementation enhances Nrf2 activation, thereby strengthening both non-enzymatic and enzymatic antioxidant defenses to mitigate oxidative stress in the intestines of piglets infected with *E. coli*. Our findings highlight the critical role that probiotics can

play in maintaining intestinal health by reducing oxidative stress, restoring gut barrier integrity, and alleviating inflammation. Previous research has indicated that probiotic supplementation modulates gut microbiota and immune responses, contributing to intestinal health (Zhou et al., 2022). In this study, *Bacillus licheniformis* HG76 not only improved antioxidant capacity by upregulating GSH synthesis but also reduced pro-inflammatory cytokine expression and restored tight-junction protein integrity. These results highlight the therapeutic potential of probiotics in mitigating oxidative damage and promoting gut health during *E. coli* infection. Further studies are warranted to uncover the precise mechanisms through which probiotics activate Nrf2 signaling and regulate glutathione synthesis and metabolism. Understanding how these interactions enhance antioxidant pathways, modulate gut microbiota composition, and influence immune homeostasis will provide deeper insights into the role of probiotics in mitigating oxidative damage and promoting intestinal health during *E. coli* infection.

In conclusion, this study demonstrates that *Bacillus licheniformis* HG76 supplementation effectively mitigates the oxidative stress and intestinal dysfunction caused by *E. coli* infection. *Bacillus licheniformis* HG76 can enhance Nrf2 activation and boost both non-enzymatic and enzymatic antioxidant defenses. These actions led to a significant reduction in oxidative damage, restoration of gut barrier integrity through increased expression of tight-junction proteins, and a decrease in inflammatory responses. This study suggests the potential of *Bacillus licheniformis* HG76 as a therapeutic intervention to strengthen antioxidant capacity, mitigate inflammation, and improve overall intestinal health during *E. coli* infection.

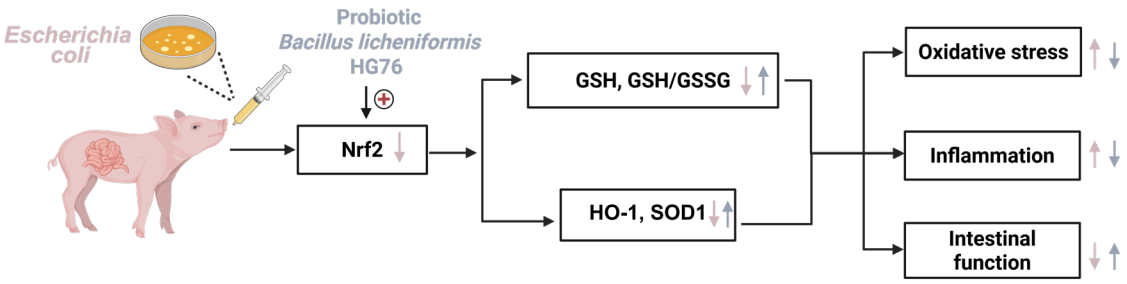

Figure 5.7 Mechanisms of protective role of *Bacillus licheniformis* HG76 in *Escherichia coli* infected oxidative stress and intestinal dysfunction

Escherichia coli infection disrupted Nrf2 signaling in piglets, reducing the expression of key antioxidant enzymes (HO-1, SOD1) and lowering GSH levels and the GSH/GSSG ratio. These changes increased oxidative stress, triggered inflammation, and weakened intestinal barrier integrity, leading to intestinal dysfunction. Supplementation with *Bacillus licheniformis* HG76 countered these effects by reactivating Nrf2 signaling, which increased antioxidant enzyme levels and restored redox balance (higher GSH and GSH/GSSG ratio). This probiotic action helped reduce oxidative damage, limit inflammation, and enhance intestinal health in the presence of *Escherichia coli*. Abbreviations: Nrf2, nuclear factor erythroid 2-related factor 2; GSH, reduced glutathione; GSSG, oxidized glutathione; HO-1, heme oxygenase-1.

CHAPTER SIX: GENERAL DISCUSSION

6.1 General discussion

The gastrointestinal tract plays a central role in maintaining overall health. It acts as a critical interface between dietary inputs, microbial populations, and host immune responses (Jensen et al., 2022; Aziz et al., 2024). Oxidative stress is characterized by an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, leading to cellular and tissue damage (Ballway and Song, 2021; Sies et al., 2022). The gastrointestinal tract is particularly vulnerable to oxidative damage due to its high metabolic activity and constant exposure to dietary and microbial antigens (Yun et al., 2022). Oxidative stress plays a pivotal role in the pathogenesis of many gastrointestinal disorders, and its regulation is critical for maintaining intestinal homeostasis (Vona et al., 2021). Dietary fiber plays a multifaceted role in gut health, not only as a nutrient source but also as a modulator of oxidative stress (Shah et al., 2020; Muscolo et al., 2024). Probiotics, as live beneficial microorganisms, contribute to gut health by modulating gut microbial populations, enhancing the host's immune response, strengthening intestinal barrier function, and alleviating oxidative stress in the gastrointestinal tract (Feng and Wang, 2020; Li et al., 2023b). Probiotic strains, such as *Bacillus* and *Lactobacillus* species, have been shown to improve gut health by modulating microbial populations, enhancing antioxidant enzyme activity, and reducing pro-inflammatory cytokines (Kong et al., 2020; Weng et al., 2025). This thesis aimed to investigate the impact of oxidative stress on intestinal health in pigs and explored potential dietary and probiotic strategies to mitigate oxidative damage. By examining the effects of dietary fiber, pathogenic challenges, and probiotic interventions, this research provided mechanistic insights and practical approaches for improving intestinal health and reducing the reliance on antibiotics in

livestock production. Our research demonstrated that:1) Under normal conditions, low-fiber diets induced oxidative stress, prompting the intestine to activate glutathione synthesis as a compensatory response, while high-fiber diets helped maintain redox balance and reduced oxidative burden; 2) During bacteria ETEC infection, oxidative stress was caused by the suppression of Nrf2 signaling and the depletion of glutathione, leading to impaired intestinal barrier integrity and increased inflammation; 3) Probiotic *Bacillus licheniformis* HG76 restored redox balance by activating Nrf2 signaling and enhancing glutathione synthesis, effectively mitigating ETEC-induced oxidative damage and protecting intestinal integrity.

6.2. Dietary fiber in livestock production

6.2.1The role of dietary fiber in modulating oxidative stress

Dietary fiber has long been recognized for its positive effects on gut health, primarily through its role in modulating microbial activity and producing short-chain fatty acids like butyrate (Gill et al., 2021). However, its specific impact on oxidative stress in pigs under normal physiological conditions requires further exploration. Dietary fiber level influences gut function not only through microbial fermentation but also by modulating antioxidant pathways, which can protect intestinal cells from oxidative damage (Han et al., 2023). Study 1 aimed to determine how high- and low-fiber levels in diets affected intestinal oxidative stress markers and antioxidant defenses, providing foundational insights into dietary modulation of redox balance. This is particularly relevant as the pork industry increasingly adopts high-fiber ingredients to reduce feed costs and improve sustainability. The study revealed that pigs on a high-fiber diet had lower levels of oxidative stress

compared to those on a low-fiber diet. Specifically, the markers of lipid peroxidation were significantly lower in the high-fiber group, suggesting that high-fiber diets helped mitigate oxidative damage in the intestinal environment. Conversely, pigs fed a low-fiber diet showed signs of oxidative stress adaptation, evidenced by increased synthesis of glutathione and its oxidized form. This indicates that the low-fiber diet imposes a higher oxidative challenge, prompting the intestine to enhance its antioxidant defenses. Interestingly, the overall redox balance, reflected in the GSH/GSSG ratio, remained stable across both groups. This suggested that while antioxidant defenses were activated under the low-fiber diet condition, the oxidative burden was inherently higher, potentially leading to long-term stress in the intestinal tissue. These results are consistent with other research showing that dietary fiber can directly influence antioxidant systems by promoting the production of beneficial metabolites like butyrate, which support the epithelial barrier and reduce oxidative damage (Khoshbin and Camilleri, 2020; Liu et al., 2021; Salazar-Bermeo et al., 2021; Munteanu and Schwartz, 2024).

6.2.2 Optimizing dietary fiber for oxidative stress management and growth performance

The impact of dietary fiber on oxidative stress was further reflected in growth performance outcomes. High-fiber diets, particularly those rich in insoluble fibers, supported better growth performance, likely due to improved gut health. In contrast, the animals fed low-fiber diets experienced reduced growth rates, potentially linked to increased oxidative stress, which could impair intestinal function and nutrient utilization. The differences observed between the two groups fed with high or low fiber content highlighted the

importance of fiber in dietary formulations. This study underscored the dual role of dietary fiber as both a preventive and adaptive factor in oxidative stress management. High-fiber diets not only reduced oxidative challenges but also spared the intestine from the need to upregulate endogenous antioxidant pathways, preserving metabolic energy for growth and maintenance. Conversely, low-fiber diets, while capable of triggering compensatory antioxidant responses, might have imposed long-term oxidative stress on the intestinal system. These results suggested that optimizing dietary fiber content was crucial for sustaining gut health and enhancing animal productivity. These findings align with the previous research emphasizing the role of dietary fiber in reducing oxidative stress through microbiota-mediated mechanisms. Those studies have shown that butyrate, derived from fiber fermentation, enhance antioxidant enzyme activity and improve epithelial barrier function (Su et al., 2022b; Zhang et al., 2024). Study 1 extended existing knowledge by demonstrating a direct link between dietary fiber levels and GSH biosynthesis pathways in pigs, which suggested that an inadequate fiber intake increased the oxidative burden at a molecular level.

6.2.3 Physicochemical properties and NSP composition of fiber sources shape their functional roles

The effects of dietary fiber supplementation in pigs depend not only on the total fiber content but also on the soluble-to-insoluble NSP composition, which influences fermentability, gut motility, and microbial interactions (Jha and Berrocoso, 2015). In the present study, the high-fiber diet, formulated with increased inclusion of oat hulls and soybean hulls, was characterized by a higher total NSP level, predominantly composed of

insoluble components such as cellulose and arabinoxylans. In contrast, the low-fiber diet, while lower in total NSP, contained a slightly higher proportion of soluble NSP, including fermentable pectins and hemicelluloses. The high-fiber diet maintained intestinal redox balance and improved growth performance, suggesting that insoluble NSP can exert positive effects on gut function when included in appropriate amounts. Insoluble fibers increase luminal bulk, stimulate intestinal motility, and enhance epithelial turnover, all of which contribute to improved nutrient absorption and barrier integrity (Kim et al., 2008; Jha et al., 2019). Moreover, our findings align with other reports indicating that moderate levels of insoluble fiber can reduce post-weaning diarrhea and promote beneficial microbial colonization, particularly of *Lactobacillus* and *Faecalibacterium* spp., thereby supporting systemic health and growth (Muramatsu and Winter, 2024). While soluble NSP can stimulate SCFA production and immune modulation, our data sugget that insoluble fiber, when strategically incorporated, can also yield significant functional benefits, particularly by improving intestinal redox homeostasis. Therefore, fiber functionality in pig diets cannot be evaluated based solely on solubility. Instead, both the type and inclusion level of NSP must be considered in combination with developmental stage and physiological context. Future research should aim to refine fiber formulations by balancing soluble and insoluble fractions to achieve optimal gut fermentation patterns, barrier function, and growth trajectories in swine production.

6.3 Exploring the impact of ETEC infection on redox homeostasis and intestinal health

While Study 1 highlighted how dietary fiber modulates oxidative stress under normal conditions, it left unexplored the effects of external stressors, such as pathogenic infections, on the intestinal antioxidant defense mechanisms. Enterotoxigenic *Escherichia coli* infection is a major cause of post-weaning diarrhea in piglets, impairing growth performance and compromising gut integrity (Eriksen et al., 2021; Wang et al., 2024b). ETEC not only disrupts the intestinal barrier but also induces oxidative stress, amplifying inflammatory response and tissue damage. However, the precise mechanisms linking oxidative stress to intestinal dysfunction during ETEC infection remained unclear. Study 2 aimed to dissect the mechanisms through which ETEC infection alters redox homeostasis and to explore how these changes contribute to gut dysfunction.

6.3.1 ETEC-induced oxidative stress: disruption of Nrf2 and intestinal barrier integrity

Study 2 demonstrated that ETEC infection caused a significant increase in oxidative stress markers, MDA, in the intestine of the infected piglets. Simultaneously, the levels of GSH, the primary intracellular antioxidant, were markedly reduced in the jejunum and serum. Such a depletion reflected a weakened antioxidant defense system that was unable to counteract the rising ROS levels effectively. Additionally, ETEC infection significantly reduced the expression of glutathione synthesizing enzymes. The suppression of these enzymes indicated a direct disruption of glutathione metabolism, exacerbating the oxidative stress. The suppression of Nrf2 signaling pathway was a central mechanism

contributing to this oxidative imbalance. The reduced nuclear level of Nrf2 observed in this study led to a decline in the expression of antioxidant enzymes in the intestine. Nrf2 is a transcription factor that regulates the expression of glutathione synthesizing enzymes. The reduction in GSH synthesis aligned with the suppression of Nrf2 observed in this study. This disruption highlights how ETEC actively impairs the host's ability to counteract ROS, leading to sustained oxidative stress. The oxidative stress induced by ETEC infection had downstream effects on intestinal barrier integrity. The decreased expression of tight junction proteins compromised intestinal barrier function, leading to increased gut permeability, as evidenced by elevated serum DAO activity and D-lactate level. This disruption of the epithelial barrier may facilitate the translocation of pathogens and toxins into the systemic circulation, exacerbating inflammation and further compounding oxidative damage. The observed changes in glutathione metabolism and Nrf2 suppression established a cause-and-effect relationship: ETEC infection reduced the expression of glutathione synthesis enzymes, depleting GSH levels and impairing antioxidant defenses. This oxidative imbalance led to lipid peroxidation, tight junction disruption, and ultimately intestinal barrier dysfunction.

6.3.2 Therapeutic potential of GSH in attenuating oxidative stress and improving gut barrier integrity

In vitro experiments demonstrated that exogenous GSH supplementation restored the expression of tight junction protein intestinal epithelial cells, demonstrating a correlation between oxidative stress and epithelial barrier function. By reversing the effects of H_2O_2-induced Nrf2 suppression, GSH improved intestinal barrier integrity, reducing epithelial

permeability and mitigating the risk of systemic inflammation. These results suggested that the disruption of glutathione metabolism was a central driver of barrier dysfunction, which could be effectively targeted through antioxidant restoration. These results provided a mechanistic link between Nrf2 suppression and impaired glutathione metabolism, distinguishing this study from earlier work on oxidative stress in bacterial infections. These findings underscored the central role of glutathione metabolism in maintaining intestinal health during bacterial infections. The ability of GSH to restore nuclear Nrf2 expression and improve both antioxidant defenses and barrier function highlighted its potential as a therapeutic agent.

6.4 Probiotics as sustainable alternatives for counteracting ETEC-induced oxidative stress

Building on the insights gained from Study 2, which demonstrated the efficacy of GSH in restoring Nrf2 expression and mitigating oxidative stress, the next step was to explore sustainable alternatives. Probiotics have gained significant attention as natural modulators of gut health, particularly under stress conditions caused by pathogenic infections (Mousavi Khaneghah et al., 2020). Their mechanisms include promoting beneficial microbial populations and producing short-chain fatty acids (Raheem et al., 2021; Rastogi and Singh, 2022). *Bacillus licheniformis*, a spore-forming probiotic, has shown potential for surviving harsh gastrointestinal environments, allowing it to directly influence gut homeostasis (Li, 2021; Todorov et al., 2022). Study 3 hypothesized that *Bacillus licheniformis* HG76 could counteract ETEC-induced oxidative stress restoring Nrf2

activity, enhancing GSH synthesizing enzyme expression, and protecting the intestinal barrier.

6.4.1 *Bacillus licheniformis* HG76: A multifaceted approach to mitigating ETEC-induced oxidative stress and gut dysfunction

Under *E. coli* infection condition, the *Bacillus licheniformis* HG76 supplement could effectively restore glutathione levels and reduce MDA, highlighting its role in enhancing the host's antioxidant capacity. This restoration was mediated by upregulation of key glutathione synthesizing enzymes. Oxidative stress during infection is closely linked to intestinal barrier dysfunction. A key mechanism underlying this effect was the activation of the Nrf2 signaling pathway. *Bacillus licheniformis* HG76 supplementation activated Nrf2, enhancing its nuclear level and restoring the expression of antioxidant enzymes. *Bacillus licheniformis* HG76 supplementation also significantly improved intestinal barrier integrity by upregulating tight junction proteins such as ZO-1 and occludin, which were otherwise reduced under stress conditions. Additionally, the probiotic reduced the markers of intestinal permeability, including serum DAO activity and D-lactate level, indicating its protective effects on gut epithelial function. Furthermore, *Bacillus licheniformis* HG76 also had anti-inflammatory effects by reducing the expression of pro-inflammatory cytokines, including IL-6, TNF-α and IL-1β. This reduction in inflammation was likely a downstream result of improved antioxidant defenses, as oxidative stress is a known driver of inflammatory pathways. By modulating both oxidative and inflammatory responses, the probiotic effectively protected the gut during stress conditions. These results underscored the multifaceted benefits of *Bacillus licheniformis* HG76 in addressing oxidative stress,

intestinal barrier dysfunction, and inflammation. While previous studies on *Bacillus* species have highlighted their general gut health benefits, this study uniquely demonstrated their ability to upregulate GSH synthesis and activate Nrf2 signaling. Moreover, these results extended previous research on probiotics by emphasizing their capacity to restore antioxidant enzyme systems in livestock.

CHAPTER SEVEN: CONCLUSIONS, LIMITATIONS AND FUTURE PERSPECTIVES

7.1 Conclusions

The present study provides significant insights into the role of oxidative stress in intestinal health. It highlights the dietary fiber and probiotic strategies to mitigate oxidative damage in pigs under physiological and pathogenic conditions, respectively. The major findings include:

1) the evidence that high-fiber diets can protect against oxidative stress, enhance GSH synthesis, and maintain intestinal homeostasis under physiological conditions, while simultaneously improving growth performance.

2) the identification of oxidative stress as a critical factor contributing to gut injury during infection, primarily through the downregulation of Nrf2 signaling pathways, which leads to impaired antioxidant defenses and increased inflammation.

3) the demonstration that GSH supplementation in the culture medium alleviated H_2O_2-induced oxidative stress in intestinal epithelial cells, which was associated with upregulated tight junction protein expression to support barrier integrity, and downregulated pro-inflammatory cytokine expression.

4) the efficacy of Bacillus licheniformis HG76 in mitigating ETEC-induced oxidative stress by reactivating Nrf2 signaling, increasing GSH synthesis, reducing oxidative damage, and restoring tight-junction protein expression to protect gut barrier integrity.

Overall, the present study has identified the critical role of oxidative stress in gut health. It has demonstrated the potential of dietary fiber and probiotic strategies to restore antioxidant defenses, reduce inflammation, and improve intestinal function. The integration of these strategies into livestock production systems represents a significant advancement toward sustainable and efficient animal management practices.

7.2 Limitations and future perspectives

7.2.1 Impact of soluble and insoluble fiber levels on intestinal oxidative stress

The first study established that fiber supplementation could maintain intestinal redox balance and improve growth performance. Dietary fiber can be divided into soluble and insoluble fiber according to its solubility in water (Wang et al., 2022c). Each type exerts distinct physiological effects on gut health, influencing digestion, microbiota composition, and intestinal function (Gill et al., 2021). Soluble fibers, such as inulin, pectin, and resistant starch, are fermentable by gut microbiota, leading to increased production of butyrate, which possesses anti-inflammatory properties (Blanco-Pérez et al., 2021; Muthyala et al., 2022; Chen et al., 2024b). These fibers also have been shown to enhance the growth of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, modulating gut microbiota composition and maintaining gut function (Wang et al., 2022a). Furthermore, different inclusion levels of soluble fiber have been reported to variably affect gut barrier function. While moderate levels enhance mucus production and improve intestinal barrier integrity, excessive fibers can lead to osmotic imbalances and excessive fermentation, causing bloating and diarrhea (Chen et al., 2022b). Insoluble fibers, primarily affect gut motility and fecal bulk rather than microbial fermentation (Baky et al., 2024). They accelerate intestinal transit time, reducing gut residence time for harmful metabolites (Baky et al., 2024). Different levels of insoluble fiber inclusion have been shown to modulate gut morphology. Moderate levels promote epithelial proliferation and support gut integrity, while excessive levels can potentially cause mechanical irritation of the mucosa, leading to inflammation (Jha and Berrocoso, 2015; Gill et al., 2021). To address the limitations in understanding fiber dose-response relationships, future studies should be designed as dos-

response trials incorporating multiple levels of both soluble and insoluble fiber types in a factorial design. Key outcome measures should include redox biomarkers (e.g., GSH:GSSG ratio, MDA), gut permeability assays (e.g., FITC-dextran), and microbial fermentation endpoints (e.g., SCFAs profiles). Advanced fiber characterization, including water-holding capacity, fermentability assays, and viscosity measurements, should be integrated into the trial design. Longitudinal sampling across multiple post-weaning time points would clarify dynamic changes in response to dietary fiber and enable better definition of optimal inclusion thresholds. Investigating the interactions between fiber type, concentration, and oxidative stress regulation will provide valuable insights for precision dietary strategies. Advanced methodologies such as metabolomics and microbiome sequencing should be employed to elucidate fiber-induced metabolic shifts and their impact on oxidative stress regulation.

7.2.2 Long-term effects of Nrf2 modulation in gut health

The second study highlighted the modulation of Nrf2 activity as a key mechanism for oxidative stress regulation during ETEC infection. In piglet production, *E. coli* infections are a major cause of post-weaning diarrhea and reduced growth performance (Canibe et al., 2022). Short-term infections often occur within the first few weeks post-weaning due to immature gut immunity, leading to acute diarrhea and transient dysbiosis (Tang et al., 2024). Studies have reported that acute *E. coli* infections in piglets increase the expression of pro-inflammatory cytokines such as IL-1β and TNF-α, and reduce villus height, impairing nutrient absorption (Kim et al., 2022b). Long-term or recurrent infections, often due to subclinical *E. coli* exposure in contaminated environments, can result in chronic gut

inflammation and persistent microbiota imbalances (Sun et al., 2020a). Long-term studies have shown that recurrent *E. coli* infections disrupt gut homeostasis by shifting microbiota composition toward pathogenic strains, reducing beneficial *Lactobacillus* populations, and causing intestinal fibrosis (Small et al., 2013; Sun et al., 2020a). These chronic infections may impair nutrient absorption, leading to stunted growth and increased susceptibility to secondary infections. Additionally, some studies have shown that individuals with a history of prolonged *E. coli* infections have a higher prevalence of inflammatory bowel disease due to persistent immune stimulation and compromised gut barrier function (Ternhag et al., 2008). To expand on this mechanism, future research should employ chronic or repeated *E. coli* challenge models over extended post-weaning periods (e.g., 4-6 weeks) to assess the sustained regulatory role of Nrf2. Experimental designs should include Nrf2 knockout or inhibitor groups to confirm causality and delineate downstream targets via transcriptomic or proteomic analysis of intestinal tissues. Additionally, integrating histological evaluation of intestinal fibrosis, immune cell infiltration, and gut permeability would provide comprehensive insight into long-term consequences of Nrf2 modulation. Understanding whether activating Nrf2 can alleviate gut damage caused by chronic oxidative stress will provide valuable insights into potential therapeutic strategies.

7.2.3 Probiotic effects on microbiota composition

The third study demonstrated the efficacy of *Bacillus licheniformis* HG76 in reducing oxidative stress and enhancing gut integrity. Probiotics have been shown to modulate microbial diversity and function, indirectly affecting antioxidant pathways (Riaz Rajoka et al., 2021). Probiotic administration can enhance the abundance of beneficial bacteria which

contribute to gut homeostasis by increasing SCFAs production and reducing the proliferation of pathogenic bacteria (Tojo et al., 2014; Surendran Nair et al., 2017; Markowiak-Kopeć and Śliżewska, 2020). Butyrate has been shown to reduce oxidative stress regulation by maintaining intestinal barrier function and modulating inflammatory responses (Liu et al., 2021). The gut microbiota plays a crucial role in controlling oxidative stress (Kunst et al., 2023). Microbial-derived metabolites, such as SCFAs and polyphenol metabolites, activate host antioxidant pathways (Munteanu and Schwartz, 2024). When the gut microbiota is balanced, it helps reduce oxidative damage and supports gut health. However, dysbiosis, or an imbalance in microbial composition, increases oxidative stress, weakens the gut barrier, and promotes inflammation (Li et al., 2023a). The specific role of *Bacillus licheniformis* HG76 in this regulatory process is still unclear and requires further investigation. Future studies should utilize gnotobiotic or antibiotic-treated piglet models to evaluate the specific contribution of *B. licheniformis* HG76 to microbiota-mediated antioxidant responses. Time-series sampling with 16S rRNA and shotgun metagenomics should be used to capture probiotic-induced microbial shifts, while concurrent measurement of SCFA levels and antioxidant gene expression (e.g., Nrf2, HO-1) will help clarify functional outcomes. Additionally, future trials should test the synergistic effects of co-administering *B. licheniformis* with known Nrf2 activators (e.g., sulforaphane) or soluble fiber to explore multi-modal strategies for redox balance.

In conclusion, the present studies demonstrate that dietary fiber, Nrf2 activation, and probiotic supplementation are effective strategies for regulating oxidative stress and maintaining intestinal health in pigs. High-fiber diets, predominantly composed of

insoluble non-starch polysaccharides, such as cellulose and arabinoxylans, improved intestinal redox balance and growth performance, highlighting the functional benefits of appropriately formulated insoluble fiber. Optimizing the balance of soluble and insoluble fiber may enhance gut barrier function and microbial fermentation, improving feed efficiency and growth performance. Nrf2 downregulation during ETEC infection compromises antioxidant defenses and exacerbates inflammation, and its role in chronic oxidative stress remains unclear. However, its long-term regulatory potential in chronic or recurrent infections remains unclear, warranting future studies using extended *E. coli* challenge models to determine whether sustained Nrf2 activation can protect the intestinal barrier and reduce post-weaning morbidity. Further research is needed to determine whether sustained Nrf2 activation can mitigate long-term oxidative damage, maintain intestinal homeostasis, and enhance resilience against recurrent infections, ultimately improving post-weaning survival and growth performance. *Bacillus licheniformis HG76* reduces oxidative stress and supports gut integrity, but whether it also exerts its antioxidant effects through gut microbiota remains unclear. Taken together, these results emphasize that dietary fiber functionality depends not only on total inclusion levels, but also on physicochemical properties, solubility, and interaction with the host microbiota and immune system. Future research should integrate dose-response designs, multi-omics approaches, and long-term infection models to refine fiber-based strategies, optimize Nrf2-targeted interventions, and unravel probiotic-host-microbiota interactions. Such integrative approaches will contribute to the development of precision nutritional solutions that enhance gut health, improve feed efficiency and growth trajectories, and ultimately reduce the reliance on antibiotics in modern swine production systems.

REFERENCES

Abdi, M., and R. Ranjbar. 2022. A review on antiviral efficacy of *Bifidobacterium* species. *Rev. Res. Med. Microbiol.* 33:74-81. doi:10.1097/MRM.0000000000000295

Adom, K. K., M. E. Sorrells, and R. H. Liu. 2003. Phytochemical profiles and antioxidant activity of wheat varieties. *J. Agric. Food. Chem*. 51:7825-7834. doi:10.1021/jf0304041

Afzal, S., A. S. Abdul Manap, A. Attiq, I. Albokhadaim, M. Kandeel, and S. M. Alhojaily. 2023. From imbalance to impairment: the central role of reactive oxygen species in oxidative stress-induced disorders and therapeutic exploration. *Front. Pharmacol.* 14:1269581. doi:10.3389/fphar.2023.1269581

Ager, E. O., T. Carvalho, E. M. Silva, S. C. Ricke, and J. L. Hite. 2023. Global trends in antimicrobial resistance on organic and conventional farms. *Sci. Rep.* 13:22608. doi:10.1038/s41598-023-47862-7

Akdis, C. A. 2021. Does the epithelial barrier hypothesis explain the increase in allergy, autoimmunity and other chronic conditions? *Nat. Rev. Immunol.* 21:739-751. doi:10.1038/s41577-021-00538-7

Akhter, N., A. Wilson, H. Arefanian, R. Thomas, S. Kochumon, F. Al-Rashed, M. Abu-Farha, A. Al-Madhoun, F. Al-Mulla, R. Ahmad, and S. Sindhu. 2023. Endoplasmic reticulum stress promotes the expression of TNF-α in THP-1 Cells by mechanisms involving ROS/CHOP/HIF-1α and MAPK/NF-κB pathways. *Int. J. Mol. Sci.* 24:15186. doi:10.3390/ijms242015186

Alula, K. M., A. S. Dowdell, B. LeBere, J. S. Lee, C. L. Levens, K. A. Kuhn, B. A. Kaipparettu, W. E. Thompson, R. S. Blumberg, S. P. Colgan, and A. L. Theiss.

2023. Interplay of gut microbiota and host epithelial mitochondrial dysfunction is necessary for the development of spontaneous intestinal inflammation in mice. *Microbiome* 11:256. doi:10.1186/s40168-023-01686-9

Amimo, J. O., S. A. Raev, J. Chepngeno, A. O. Mainga, Y. Guo, L. Saif, and A. N. Vlasova. 2021. Rotavirus interactions with host intestinal epithelial cells. *Front. Immunol.* 12:793841. doi:10.3389/fimmu.2021.793841

Anjum, N. A., A. Sofo, A. Scopa, A. Roychoudhury, S. S. Gill, M. Iqbal, A. S. Lukatkin, E. Pereira, A. C. Duarte, and I. Ahmad. 2015. Lipids and proteins-major targets of oxidative modifications in abiotic stressed plants. *Environ. Sci. Pollut. Res.* 22:4099-4121. doi:10.1007/s11356-014-3917-1

Ankom Technology. 2006a. *Acid detergent fiber in feeds filter bag technique (for A2000, A2000I). Ankom Technology Method 8.*Ankom Technology Macedon, NY.

Ankom Technology. 2006b. *Neutral detergent fiber in feeds-filter bag technique (for A2000 and A2000I). Ankom Technology Method 9.* Ankom Technology Corp Macedon, NY.

Anthone, G. J., M. S. Orandle, Z. V. Mavrophilipos, B. H. Wang, and C. J. Yeo. 1991. Neuropeptide Y is a proabsorptive agent in the small intestine. *J. Surg. Res.* 50:589-594. doi:10.1016/0022-4804(91)90047-P

AOAC International. 2000. *Official methods of analysis.*17th ed. Arlington (VA): Assoc. Off. Anal. Chem.

AOCS. 2009a. Approved Procedure Am 5-04: Rapid determination of oil/fat utilizing high temperature solvent extraction. In: D. Firestone, editor, *Official methods and*

recommended practices. 4th ed. Champaign (IL): American Oil Chemists' Society. pp. 8-53.

AOCS. 2009b. Approved Procedure Ba 6a-05: crude fiber analysis in feeds by filter bag technique. In: D. Firestone, editor, *Officials Methods and Recommended Practices*. 4th ed. Champaign (IL): American Oil Chemists' Society;. pp. 67-105.

Arbizu, S., B. Chew, S. U. Mertens-Talcott, and G. Noratto. 2020. Commercial whey products promote intestinal barrier function with glycomacropeptide enhanced activity in downregulating bacterial endotoxin lipopolysaccharides (LPS)-induced inflammation in vitro. *Food Funct.* 11:5842-5852. doi:10.1039/D0FO00487A

Arcuni, J. C., M. C. Stoner, and J. M. Kellum. 2000. Vasoactive intestinal peptide is a neuropeptide mediator of the secretory response to serotonin in rat. *J. Surg. Res.* 91:118-122. doi:10.1006/jsre.2000.5915

Arthi, V., and E. B. Schneider. 2021. Infant feeding and post-weaning health: evidence from turn-of-the-century London. *Econ. Hum. Biol.* 43:101065. doi:10.1016/j.ehb.2021.101065

Asadpoor, M., G.-N. Ithakisiou, P. A. J. Henricks, R. Pieters, G. Folkerts, and S. Braber. 2021. Non-digestible oligosaccharides and short chain fatty acids as therapeutic targets against enterotoxin-producing bacteria and their toxins. *Toxins* 13:175. doi:10.3390/toxins13030175

Au-Yeung, K. K. W., Y. Shang, C. U. B. Wijerathne, S. Madduma Hewage, Y. L. Siow, and K. O. 2023. Acute kidney injury induces oxidative stress and hepatic lipid accumulation through AMPK signaling pathway. *Antioxidants* 12:883. doi:10.3390/antiox12040883

Aviello, G., and U. G. Knaus. 2017. ROS in gastrointestinal inflammation: rescue or sabotage? *Br. J. Pharmacol.* 174:1704-1718. doi:10.1111/bph.13428

Aziz, T., N. Hussain, Z. Hameed, and L. Lin. 2024. Elucidating the role of diet in maintaining gut health to reduce the risk of obesity, cardiovascular and other age-related inflammatory diseases: recent challenges and future recommendations. *Gut Microbes* 16:2297864. doi:10.1080/19490976.2023.2297864

Bailén, M., C. Bressa, S. Martínez-López, R. González-Soltero, M. G. Montalvo Lominchar, C. San Juan, and M. Larrosa. 2020. Microbiota features associated with a high-fat/low-fiber diet in healthy adults. *Front. Nutr.* 7:583608. doi:10.3389/fnut.2020.583608

Baker, A., C.-C. Lin, C. Lett, B. Karpinska, M. H. Wright, and C. H. Foyer. 2023. Catalase: A critical node in the regulation of cell fate. *Free Radic. Biol. Med.* 199:56-66. doi:10.1016/j.freeradbiomed.2023.02.009

Baky, M. H., M. Salah, N. Ezzelarab, P. Shao, M. S. Elshahed, and M. A. Farag. 2024. Insoluble dietary fibers: structure, metabolism, interactions with human microbiome, and role in gut homeostasis. *Crit. Rev. Food Sci. Nutr.* 64:1954-1968. doi:10.1080/10408398.2022.2119931

Baldelli, V., F. Scaldaferri, L. Putignani, and F. Del Chierico. 2021. The role of Enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. *Microorganisms* 9:697. doi:10.3390/microorganisms9040697

Ballway, J. W., and B.-J. Song. 2021. Translational approaches with antioxidant phytochemicals against alcohol-mediated oxidative stress, gut dysbiosis, intestinal

barrier dysfunction, and fatty liver disease. *Antioxidants* 10:384. doi:10.3390/antiox10030384

Bany Bakar, R., F. Reimann, and F. M. Gribble. 2023. The intestine as an endocrine organ and the role of gut hormones in metabolic regulation. *Nat. Rev. Gastroenterol. Hepatol.* 20:784-796. doi:10.1038/s41575-023-00830-y

Barbara, G., M. R. Barbaro, D. Fuschi, M. Palombo, F. Falangone, C. Cremon, G. Marasco, and V. Stanghellini. 2021. Inflammatory and microbiota-related regulation of the intestinal epithelial barrier. *Front. Nutr.* 8:718356. doi:10.3389/fnut.2021.718356

Bausys, A., J. Maneikyte, B. Leber, J. Weber, N. Feldbacher, K. Strupas, T. B. Dschietzig, P. Schemmer, and P. Stiegler. 2021. Custodiol® supplemented with synthetic human relaxin decreases ischemia-reperfusion injury after porcine kidney transplantation. *Int. J. Mol. Sci.* 22:11417. doi:10.3390/ijms222111417

Bearcroft, C. P., D. Perrett, and M. J. Farthing. 1996. 5-hydroxytryptamine release into human jejunum by cholera toxin. *Gut* 39:528. doi:10.1136/gut.39.4.528

Bellezza, I., I. Giambanco, A. Minelli, and R. Donato. 2018. Nrf2-Keap1 signaling in oxidative and reductive stress. *Biochim. Biophys. Acta, Mol. Cell Res.* 1865:721-733. doi:10.1016/j.bbamcr.2018.02.010

Bested, A. C., A. C. Logan, and E. M. Selhub. 2013. Intestinal microbiota, probiotics and mental health: from Metchnikoff to modern advances: Part II - contemporary contextual research. *Gut Pathog.* 5:3. doi:10.1186/1757-4749-5-3

Betancur, C., Y. Martínez, R. Merino-Guzman, X. Hernandez-Velasco, R. Castillo, R. Rodríguez, and G. Tellez-Isaias. 2020. Evaluation of oral administration of

Lactobacillus plantarum CAM6 strain as an alternative to antibiotics in weaned pigs. *Animals* 10:1218. doi:10.3390/ani10071218

Bevins, C. L., and N. H. Salzman. 2011. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat. Rev. Microbiol.* 9:356-368. doi:10.1038/nrmicro2546

Bhat, M. I., K. Sowmya, S. Kapila, and R. Kapila. 2020. Potential probiotic *Lactobacillus rhamnosus* (MTCC-5897) inhibits *Escherichia coli* impaired intestinal barrier function by modulating the host tight junction gene response. *Probiotics Antimicrob. Proteins* 12:1149-1160. doi:10.1007/s12602-019-09608-8

Biernbaum, E. N., and I. T. Kudva. 2022. AB5 Enterotoxin-Mediated Pathogenesis: Perspectives Gleaned from Shiga Toxins. *Toxins* 14:doi:10.3390/toxins14010062

Bilal, M., S. Ashraf, and X. Zhao. 2022. Dietary component-induced inflammation and its amelioration by prebiotics, probiotics, and synbiotics. *Front. Nutr.* 9:931458. doi:10.3389/fnut.2022.931458

Bin, P., Z. Tang, S. Liu, S. Chen, Y. Xia, J. Liu, H. Wu, and G. Zhu. 2018. Intestinal microbiota mediates enterotoxigenic *Escherichia coli*-induced diarrhea in piglets. *BMC Vet. Res.* 14:385. doi:10.1186/s12917-018-1704-9

Blachier, F., M. Andriamihaja, P. Larraufie, E. Ahn, A. Lan, and E. Kim. 2020. Production of hydrogen sulfide by the intestinal microbiota and epithelial cells and consequences for the colonic and rectal mucosa. *Am. J. Physiol. Gastrointest. Liver Physiol.* 320:G125-G135. doi:10.1152/ajpgi.00261.2020

Blanco-Pérez, F., H. Steigerwald, S. Schülke, S. Vieths, M. Toda, and S. Scheurer. 2021. The dietary fiber pectin: health benefits and potential for the treatment of allergies

by modulation of gut microbiota. *Curr. Allergy Asthma Rep.* 21:43. doi:10.1007/s11882-021-01020-z

Bourgonje, A. R., M. Feelisch, K. N. Faber, A. Pasch, G. Dijkstra, and H. van Goor. 2020. Oxidative stress and redox-modulating therapeutics in inflammatory bowel disease. *Trends Mol. Med.* 26:1034-1046. doi:10.1016/j.molmed.2020.06.006

Braian, C., L. Karlsson, J. Das, and M. Lerm. 2023. Selected β-Glucans act as immune-training agents by improving anti-mycobacterial activity in human macrophages: a pilot study. *J. Innate Immun.* 15:751-764. doi:10.1159/000533873

Brigelius-Flohé, R., and M. Maiorino. 2013. Glutathione peroxidases. *Biochim. Biophys. Acta, Gen. Subj.* 1830:3289-3303. doi:10.1016/j.bbagen.2012.11.020

Bron, P. A., M. Kleerebezem, R.-J. Brummer, P. D. Cani, A. Mercenier, T. T. MacDonald, C. L. Garcia-Ródenas, and J. M. Wells. 2017. Can probiotics modulate human disease by impacting intestinal barrier function? *Br. J. Nutr.* 117:93-107. doi:10.1017/S0007114516004037

Brown, K., R. R. E. Uwiera, M. L. Kalmokoff, S. P. J. Brooks, and G. D. Inglis. 2017. Antimicrobial growth promoter use in livestock: a requirement to understand their modes of action to develop effective alternatives. *Int. J. Antimicrob. Agents* 49:12-24. doi:10.1016/j.ijantimicag.2016.08.006

Buffie, C. G., and E. G. Pamer. 2013. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* 13:790-801. doi:10.1038/nri3535

Campbell, E. L., and S. P. Colgan. 2019. Control and dysregulation of redox signalling in the gastrointestinal tract. *Nat. Rev. Gastroenterol. Hepatol.* 16:106-120. doi:10.1038/s41575-018-0079-5

Canibe, N., O. Højberg, H. Kongsted, D. Vodolazska, C. Lauridsen, T. S. Nielsen, and A. A. Schönherz. 2022. Review on preventive measures to reduce post-weaning diarrhoea in piglets. *Animals* 12:2585. doi:10.3390/ani12192585

Cao, S., C. Wang, J. Yan, X. Li, J. Wen, and C. Hu. 2020. Curcumin ameliorates oxidative stress-induced intestinal barrier injury and mitochondrial damage by promoting Parkin dependent mitophagy through AMPK-TFEB signal pathway. *Free Radic. Biol. Med.* 147:8-22. doi:10.1016/j.freeradbiomed.2019.12.004

Castillo Zuniga, J., A. M. Fresno Rueda, R. S. Samuel, B. St-Pierre, and C. L. Levesque. 2024. Impact of *Lactobacillus*- and *Bifidobacterium*-based direct-fed microbials on the performance, intestinal morphology, and fecal bacterial populations of nursery pigs. *Microorganisms* 12:1786. doi:10.3390/microorganisms12091786

Catalá, A., and M. Díaz. 2016. Impact of lipid peroxidation on the physiology and pathophysiology of cell membranes. *Front. Physiol.* 7:423. doi:10.3389/fphys.2016.00423

Catalkaya, G., K. Venema, L. Lucini, G. Rocchetti, D. Delmas, M. Daglia, A. De Filippis, H. Xiao, J. L. Quiles, J. Xiao, and E. Capanoglu. 2020. Interaction of dietary polyphenols and gut microbiota: Microbial metabolism of polyphenols, influence on the gut microbiota, and implications on host health. *Food Front.* 1:109-133. doi:10.1002/fft2.25

CCAC. 2009. *Canadian Council on Animal Care guideline on: the care and use of farm animals in research, teaching and testing.*Ottawa (ON): CCAC.

Celi, P., A. J. Cowieson, F. Fru-Nji, R. E. Steinert, A. M. Kluenter, and V. Verlhac. 2017. Gastrointestinal functionality in animal nutrition and health: New opportunities for

sustainable animal production. *Anim. Feed Sci. Technol.* 234:88-100. doi:10.1016/j.anifeedsci.2017.09.012

Chai, Y.-C., and J. J. Mieyal. 2023. Glutathione and glutaredoxin-key players in cellular redox homeostasis and signaling. *Antioxidants* 12:1553. doi:10.3390/antiox12081553

Chami, B., N. J. J. Martin, J. M. Dennis, and P. K. Witting. 2018. Myeloperoxidase in the inflamed colon: a novel target for treating inflammatory bowel disease. *Arch. Biochem. Biophys.* 645:61-71. doi:10.1016/j.abb.2018.03.012

Chang, S. Y., J. H. Lee, H. J. Oh, J. W. An, D. C. Song, H. A. Cho, S. H. Park, K. H. Jeon, S. Y. Cho, D. J. Kim, M. S. Kim, and J. H. Cho. 2023. Effect of different ratios of phytogenic feed additives on growth performance, nutrient digestibility, intestinal barrier integrity, and immune response in weaned pigs challenged with a pathogenic *Escherichia coli*. *J. Anim. Sci.* 101:skad148. doi:10.1093/jas/skad148

Chang, S. Y., M. H. Song, J. H. Lee, H. J. Oh, Y. J. Kim, J. W. An, Y. B. Go, D. C. Song, H. A. Cho, S. Y. Cho, D. J. Kim, M. S. Kim, H. B. Kim, and J. H. Cho. 2022. Phytogenic feed additives alleviate pathogenic *Escherichia coli*-induced intestinal damage through improving barrier integrity and inhibiting inflammation in weaned pigs. *J. Anim. Sci. Biotechnol.* 13:107. doi:10.1186/s40104-022-00750-y

Chanin, R. B., M. G. Winter, L. Spiga, E. R. Hughes, W. Zhu, S. J. Taylor, A. Arenales, C. C. Gillis, L. Büttner, A. G. Jimenez, M. P. Smoot, R. L. Santos, and S. E. Winter. 2020. Epithelial-derived reactive oxygen species enable AppBCX-mediated aerobic respiration of *Escherichia coli* during intestinal inflammation. *Cell Host Microbe* 28:780-788. doi:10.1016/j.chom.2020.09.005

Chechet, O. N., O. I. Gorbatyuk, R. I. O, N. V. Kuryata, G. A. Buchkovska, I. V. Musiets, N. V. Shchur, L. O. Shalimova, D. O. Ordynska, L. V. Balanchuk, and L. V. Togachynska. 2023. Zoonotic and commensal bacteria from pigs with acquired antimicrobial resistance. *Regul. Mech. Biosyst.* 14:624-629. doi:10.15421/022390

Chen, F., Q. Wang, M. Xiao, D. Lou, R. Wufur, S. Hu, Z. Zhang, Y. Wang, and Y. Zhang. 2024a. A novel crosstalk between Nrf2 and Smad2/3 bridged by two nuanced Keap1 isoforms with their divergent effects on these distinct family transcription factors. *Free Radic. Biol. Med.* 213:190-207. doi:10.1016/j.freeradbiomed.2024.01.025

Chen, J., H. Pang, L. Wang, C. Ma, G. Wu, Y. Liu, Y. Guan, M. Zhang, G. Qin, and Z. Tan. 2022a. Bacteriocin-producing lactic acid bacteria strains with antimicrobial activity screened from Bamei pig feces. *Foods* 11:709. doi:10.3390/foods11050709

Chen, K., S. Man, H. Wang, C. Gao, X. Li, L. Liu, H. Wang, Y. Wang, and F. Lu. 2022b. Dysregulation of intestinal flora: excess prepackaged soluble fibers damage the mucus layer and induce intestinal inflammation. *Food Funct.* 13:8558-8571. doi:10.1039/D2FO01884E

Chen, T., D. Chen, G. Tian, P. Zheng, X. Mao, J. Yu, J. He, Z. Huang, Y. Luo, J. Luo, and B. Yu. 2019. Soluble fiber and insoluble fiber regulate colonic microbiota and barrier function in a piglet model. *Biomed Res. Int.* 2019:7809171. doi:10.1155/2019/7809171

Chen, T., D. Chen, G. Tian, P. Zheng, X. Mao, J. Yu, J. He, Z. Huang, Y. Luo, J. Luo, and B. Yu. 2020. Effects of soluble and insoluble dietary fiber supplementation on growth performance, nutrient digestibility, intestinal microbe and barrier function

in weaning piglet. *Anim. Feed Sci. Technol.* 260:114335. doi:10.1016/j.anifeedsci.2019.114335

Chen, W., H. Zhao, and Y. Li. 2023a. Mitochondrial dynamics in health and disease: mechanisms and potential targets. *Signal Transduct. Target. Ther.* 8:333. doi:10.1038/s41392-023-01547-9

Chen, X., W. Liu, J. Zhang, H. Li, and X. Liu. 2023b. Selenium-enriched peptides identified from selenium-enriched soybean protein hydrolysate: protective effects against heat damage in Caco-2 cells. *Food Funct.* 14:7882-7896. doi:10.1039/D3FO01103H

Chen, Y. P., Y. F. Gu, H. R. Zhao, and Y. M. Zhou. 2021a. Dietary squalene supplementation alleviates diquat-induced oxidative stress and liver damage of broiler chickens. *Poult. Sci.* 100:100919. doi:10.1016/j.psj.2020.12.017

Chen, Z., N. Liang, H. Zhang, H. Li, J. Guo, Y. Zhang, Y. Chen, Y. Wang, and N. Shi. 2024b. Resistant starch and the gut microbiome: Exploring beneficial interactions and dietary impacts. *Food Chem.: X* 21:101118. doi:10.1016/j.fochx.2024.101118

Chen, Z., L. Zhou, Q. Yuan, H. Chen, H. Lei, and J. Su. 2021b. Effect of fumonisin B1 on oxidative stress and gene expression alteration of nutrient transporters in porcine intestinal cells. *J. Biochem. Mol. Toxicol.* 35:e22706. doi:10.1002/jbt.22706

Choi, J., L. Wang, S. Liu, P. Lu, X. Zhao, H. Liu, L. Lahaye, E. Santin, S. Liu, M. Nyachoti, and C. Yang. 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. *J. Anim. Sci.* 98:skaa259. doi:10.1093/jas/skaa259

Circu, M. L., and T. Y. Aw. 2012. Intestinal redox biology and oxidative stress. *Semin. Cell Dev. Biol.* 23:729-737. doi:10.1016/j.semcdb.2012.03.014

Cochet, F., and F. Peri. 2017. The role of carbohydrates in the lipopolysaccharide (LPS)/toll-like receptor 4 (TLR4) signalling. *Int. J. Mol. Sci.* 18:2318. doi:10.3390/ijms18112318

Da Silva, C., C. Wagner, J. Bonnardel, J.-P. Gorvel, and H. Lelouard. 2017. The Peyer's patch mononuclear phagocyte system at steady state and during infection. *Front. Immunol.* 8:1254. doi:10.3389/fimmu.2017.01254

Dagdeviren, S., R. T. Lee, and N. Wu. 2022. Physiological and pathophysiological roles of thioredoxin interacting protein: a perspective on redox inflammation and metabolism. *Antioxid. Redox Signaling* 38:442-460. doi:10.1089/ars.2022.0022

Dahmer, P. L., O. L. Harrison, and C. K. Jones. 2022. Effects of formic acid and glycerol monolaurate on weanling pig growth performance, fecal consistency, fecal microbiota, and serum immunity. *Transl. Anim. Sci.* 6:txac145. doi:10.1093/tas/txac145

Daisley, B. A., D. Koenig, K. Engelbrecht, L. Doney, K. Hards, K. F. Al, G. Reid, and J. P. Burton. 2021. Emerging connections between gut microbiome bioenergetics and chronic metabolic diseases. *Cell Rep.* 37:doi:10.1016/j.celrep.2021.110087

de Almeida, A. J. P. O., J. C. P. L. de Oliveira, L. V. da Silva Pontes, J. F. de Souza Júnior, T. A. F. Gonçalves, S. H. Dantas, M. S. de Almeida Feitosa, A. O. Silva, and I. A. de Medeiros. 2022. ROS: Basic concepts, sources, cellular signaling, and its implications in aging pathways. *Oxid. Med. Cell. Longevity* 2022:1225578. doi:10.1155/2022/1225578

de Haan, J. B., P. J. Crack, N. Flentjar, R. C. Iannello, P. J. Hertzog, and I. Kola. 2003. An imbalance in antioxidant defense affects cellular function: the pathophysiological consequences of a reduction in antioxidant defense in the glutathione peroxidase-1 (Gpx1) knockout mouse. *Redox Rep.* 8:69-79. doi:10.1179/135100003125001378

De Santis, S., E. Cavalcanti, M. Mastronardi, E. Jirillo, and M. Chieppa. 2015. Nutritional keys for intestinal barrier modulation. *Front. Immunol.* 6:612. doi:10.3389/fimmu.2015.00612

Deehan, E. C., V. Mocanu, and K. L. Madsen. 2024. Effects of dietary fibre on metabolic health and obesity. *Nat. Rev. Gastroenterol. Hepatol.* 21:301-318. doi:10.1038/s41575-023-00891-z

Deets, K. A., and R. E. Vance. 2021. Inflammasomes and adaptive immune responses. *Nat. Immunol.* 22:412-422. doi:10.1038/s41590-021-00869-6

Degroote, J., H. Vergauwen, W. Wang, C. Van Ginneken, S. De Smet, and J. Michiels. 2020. Changes of the glutathione redox system during the weaning transition in piglets, in relation to small intestinal morphology and barrier function. *J. Anim. Sci. Biotechnol.* 11:45. doi:10.1186/s40104-020-00440-7

Deng, F., and Y. H. Bae. 2020. Bile acid transporter-mediated oral drug delivery. *J. Controlled Release* 327:100-116. doi:10.1016/j.jconrel.2020.07.034

Deng, Z., H. Yu, Z. Yang, L. Hu, Q. Liu, Y. Wang, H.-K. Wei, and J. Peng. 2021. Gly-pro-ala peptide and FGSHF3 exert protective effects in DON-induced toxicity and intestinal damage via decreasing oxidative stress. *Food Res. Int.* 139:109840. doi:10.1016/j.foodres.2020.109840

Diana, A., L. A. Boyle, F. C. Leonard, C. Carroll, E. Sheehan, D. Murphy, and E. G. Manzanilla. 2019. Removing prophylactic antibiotics from pig feed: how does it affect their performance and health? *BMC Vet. Res.* 15:67. doi:10.1186/s12917-019-1808-x

Dmytriv, T. R., K. B. Storey, and V. I. Lushchak. 2024. Intestinal barrier permeability: the influence of gut microbiota, nutrition, and exercise. *Front. Physiol.* 15:1380713. doi:10.3389/fphys.2024.1380713

Dolan, B., A. Ermund, B. Martinez-Abad, M. E. V. Johansson, and G. C. Hansson. 2022. Clearance of small intestinal crypts involves goblet cell mucus secretion by intracellular granule rupture and enterocyte ion transport. *Sci. Signaling* 15:eabl5848. doi:10.1126/scisignal.abl5848

Dong, J., L. Ping, T. Cao, L. Sun, D. Liu, S. Wang, G. Huo, and B. Li. 2022. Immunomodulatory effects of the *Bifidobacterium longum* BL-10 on lipopolysaccharide-induced intestinal mucosal immune injury. *Front. Immunol.* 13:947755. doi:10.3389/fimmu.2022.947755

Duan, Q., B. Yu, Z. Huang, Y. Luo, P. Zheng, X. Mao, J. Yu, J. Luo, H. Yan, and J. He. 2022. Protective effect of sialyllactose on the intestinal epithelium in weaned pigs upon enterotoxigenic *Escherichia coli* challenge. *Food Funct.* 13:11627-11637. doi:10.1039/D2FO02066A

Duarte, M. E., and S. W. Kim. 2022. Intestinal microbiota and its interaction to intestinal health in nursery pigs. *Anim. Nutr.* 8:169-184. doi:10.1016/j.aninu.2021.05.001

Englyst, H. N., M. E. Quigley, and G. J. Hudson. 1994. Determination of dietary fibre as non-starch polysaccharides with gas–liquid chromatographic, high-performance

liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* 119:1497-1509. doi:10.1039/AN9941901497

Eriksen, E. Ø., E. Kudirkiene, A. E. Christensen, M. V. Agerlin, N. R. Weber, A. Nødtvedt, J. P. Nielsen, K. T. Hartmann, L. Skade, L. E. Larsen, K. Pankoke, J. E. Olsen, H. E. Jensen, and K. S. Pedersen. 2021. Post-weaning diarrhea in pigs weaned without medicinal zinc: risk factors, pathogen dynamics, and association to growth rate. *Porcine Health Manage.* 7:54. doi:10.1186/s40813-021-00232-z

Fehervari, Z., and H. Kiyono. 2008. The mucosa: at the frontlines of immunity. *Trends Immunol.* 29:503-504. doi:10.1016/j.it.2008.09.001

Feng, T., and J. Wang. 2020. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes* 12:1801944. doi:10.1080/19490976.2020.1801944

Figueira, T. R., M. H. Barros, A. A. Camargo, R. F. Castilho, J. C. B. Ferreira, A. J. Kowaltowski, F. E. Sluse, N. C. Souza-Pinto, and A. E. Vercesi. 2012. Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. *Antioxid. Redox Signaling* 18:2029-2074. doi:10.1089/ars.2012.4729

Finamore, A., M. Roselli, A. Imbinto, J. Seeboth, I. P. Oswald, and E. Mengheri. 2014. *Lactobacillus amylovorus* inhibits the TLR4 inflammatory signaling triggered by enterotoxigenic *Escherichia coli* via modulation of the negative regulators and involvement of TLR2 in intestinal Caco-2 cells and pig explants. *PLoS One* 9:e94891. doi:10.1371/journal.pone.0094891

Fontenot, J., L. Smith, and A. Sutton. 1983. Alternative utilization of animal wastes. *J. Anim. Sci.* 57:221-233. doi:10.2527/animalsci1983.57Supplement_2221x

Forman, H. J., and H. Zhang. 2021. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nat. Rev. Drug Discovery* 20:689-709. doi:10.1038/s41573-021-00233-1

Foster-Nyarko, E., and M. J. Pallen. 2022. The microbial ecology of *Escherichia coli* in the vertebrate gut. *FEMS Microbiol. Rev.* 46:fuac008. doi:10.1093/femsre/fuac008

Franco, R., and J. A. Cidlowski. 2009. Apoptosis and glutathione: beyond an antioxidant. *Cell Death Differ.* 16:1303-1314. doi:10.1038/cdd.2009.107

Fukui, H. 2016. Increased intestinal permeability and decreased barrier function: does it really influence the risk of inflammation? *Inflamm. Intest. Dis.* 1:135-145. doi:10.1159/000447252

García-Sánchez, A., A. G. Miranda-Díaz, and E. G. Cardona-Muñoz. 2020. The role of oxidative stress in physiopathology and pharmacological treatment with pro-and antioxidant properties in chronic diseases. *Oxid. Med. Cell. Longevity* 2020:2082145. doi:10.1155/2020/2082145

Gardiner, G. E., B. U. Metzler-Zebeli, and P. G. Lawlor. 2020. Impact of intestinal microbiota on growth and feed efficiency in pigs: a review. *Microorganisms* 8:1886. doi:10.3390/microorganisms8121886

Geertsema, S., A. R. Bourgonje, R. R. Fagundes, R. Gacesa, R. K. Weersma, H. van Goor, G. E. Mann, G. Dijkstra, and K. N. Faber. 2023. The NRF2/Keap1 pathway as a therapeutic target in inflammatory bowel disease. *Trends Mol. Med.* 29:830-842. doi:10.1016/j.molmed.2023.07.008

George, P. 1947. Reaction between catalase and hydrogen peroxide. *Nature* 160:41-43. doi:10.1038/160041a0

Georgiou-Siafis, S. K., and A. S. Tsiftsoglou. 2023. The key role of GSH in keeping the redox balance in mammalian cells: mechanisms and significance of GSH in detoxification via formation of conjugates. *Antioxidants* 12:1953. doi:10.3390/antiox12111953

Gerritsen, R., P. van Der Aar, and F. Molist. 2012. Insoluble nonstarch polysaccharides in diets for weaned piglets. *J. Anim. Sci.* 90:318-320. doi:10.2527/jas.53770

Ghosh, S., C. S. Whitley, B. Haribabu, and V. R. Jala. 2021. Regulation of intestinal barrier function by microbial metabolites. *Cell. Mol. Gastroenterol. Hepatol.* 11:1463-1482. doi:10.1016/j.jcmgh.2021.02.007

Ghosh, S. S., J. Wang, P. J. Yannie, and S. Ghosh. 2020. Intestinal barrier dysfunction, LPS translocation, and disease development. *J. Endocr. Soc.* 4:bvz039. doi:10.1210/jendso/bvz039

Gill, S. K., M. Rossi, B. Bajka, and K. Whelan. 2021. Dietary fibre in gastrointestinal health and disease. *Nat. Rev. Gastroenterol. Hepatol.* 18:101-116. doi:10.1038/s41575-020-00375-4

Girard, C. L., H. Lapierre, A. Desrochers, C. Benchaar, J. Jacques Matte, and D. Rémond. 2001. Net flux of folates and vitamin B12 through the gastrointestinal tract and the liver of lactating dairy cows. *Br. J. Nutr.* 86:707-715. doi:10.1079/BJN2001472

Giustarini, D., A. Milzani, I. Dalle-Donne, and R. Rossi. 2023. How to increase cellular glutathione. *Antioxidants* 12:1094. doi:10.3390/antiox12051094

Goel, A., C. M. Ncho, and Y.-H. Choi. 2021. Regulation of gene expression in chickens by heat stress. *J. Anim. Sci. Biotechnol.* 12:11. doi:10.1186/s40104-020-00523-5

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

Gray, G. M. 1992. Starch digestion and absorption in nonruminants. *J. Nutr.* 122:172-177. doi:10.1093/jn/122.1.172

Grishina, O., K. Volant, F. Marquet, D. Pansu, and M. Descroix-Vagne. 1998. Effect of serotonin on unidirectional ion fluxes in rat intestine in vivo. *Gastroenterol. Clin. Biol.* 22:792-800. doi:PMID: 9854204

Guo, Y., H. Li, Z. Liu, C. Li, Y. Chen, C. Jiang, Y. Yu, and Z. Tian. 2019. Impaired intestinal barrier function in a mouse model of hyperuricemia. *Mol. Med. Rep.* 20:3292-3300. doi:10.3892/mmr.2019.10586

Gurumallesh, P., K. Alagu, B. Ramakrishnan, and S. Muthusamy. 2019. A systematic reconsideration on proteases. *Int. J. Biol. Macromol.* 128:254-267. doi:10.1016/j.ijbiomac.2019.01.081

Gustafsson, J. K., and M. E. V. Johansson. 2022. The role of goblet cells and mucus in intestinal homeostasis. *Nat. Rev. Gastroenterol. Hepatol.* 19:785-803. doi:10.1038/s41575-022-00675-x

Hageman, J. H., M. C. Heinz, K. Kretzschmar, J. van der Vaart, H. Clevers, and H. J. G. Snippert. 2020. Intestinal regeneration: regulation by the microenvironment. *Dev. Cell* 54:435-446. doi:10.1016/j.devcel.2020.07.009

Hajam, Y. A., R. Rani, S. Y. Ganie, T. A. Sheikh, D. Javaid, S. S. Qadri, S. Pramodh, A. Alsulimani, M. F. Alkhanani, and S. Harakeh. 2022. Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. *Cells* 11:552. doi:10.3390/cells11030552

Hall, M. 2000. Calculation of non-structural carbohydrate content of feeds that contain non-protein nitrogen. Gainesville: University of Florida, 2000. p. A-25.

Halliwell, B. 2024. Understanding mechanisms of antioxidant action in health and disease. *Nat. Rev. Mol. Cell Biol.* 25:13-33. doi:10.1038/s41580-023-00645-4

Han, H., Z. Liu, J. Yin, J. Gao, L. He, C. Wang, R. Hou, X. He, G. Wang, and T. Li. 2021. D-Galactose induces chronic oxidative stress and alters gut microbiota in weaned piglets. *Front. Physiol.* 12:634283. doi:10.3389/fphys.2021.634283

Han, X., Y. Ma, S. Ding, J. Fang, and G. Liu. 2023. Regulation of dietary fiber on intestinal microorganisms and its effects on animal health. *Anim. Nutr.* 14:356-369. doi:10.1016/j.aninu.2023.06.004

Harnik, Y., O. Yakubovsky, R. Hoefflin, R. Novoselsky, K. Bahar Halpern, T. Barkai, Y. Korem Kohanim, A. Egozi, O. Golani, Y. Addadi, M. Kedmi, T. Keidar Haran, Y. Levin, A. Savidor, H. Keren-Shaul, C. Mayer, N. Pencovich, R. Pery, D. S. Shouval, I. Tirosh, I. Nachmany, and S. Itzkovitz. 2024. A spatial expression atlas of the adult human proximal small intestine. *Nature* 632:1101-1109. doi:10.1038/s41586-024-07793-3

Hayhoe, M.-A. N., T. Archbold, Q. Wang, X. Yang, and M. Z. Fan. 2022. Prebiotics and β-Glucan as gut modifier feed additives in modulation of growth performance,

protein utilization status and dry matter and lactose digestibility in weanling pigs. *Front. Anim. Sci.* 3:doi:10.3389/fanim.2022.855846

Hays, K. E., J. M. Pfaffinger, and R. Ryznar. 2024. The interplay between gut microbiota, short-chain fatty acids, and implications for host health and disease. *Gut Microbes* 16:2393270. doi:10.1080/19490976.2024.2393270

He, L., T. He, S. Farrar, L. Ji, T. Liu, and X. Ma. 2017. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell. Physiol. Biochem.* 44:532-553. doi:10.1159/000485089

Hedemann, M. S., M. Eskildsen, H. N. Lærke, C. Pedersen, J. E. Lindberg, P. Laurinen, and K. E. B. Knudsen. 2006. Intestinal morphology and enzymatic activity in newly weaned pigs fed contrasting fiber concentrations and fiber properties. *J. Anim. Sci.* 84:1375-1386. doi:10.2527/2006.8461375x

Hohman, L. S., and L. C. Osborne. 2022. A gut-centric view of aging: Do intestinal epithelial cells contribute to age-associated microbiota changes, inflammaging, and immunosenescence? *Aging Cell* 21:e13700. doi:10.1111/acel.13700

Honzawa, Y., H. Nakase, M. Matsuura, and T. Chiba. 2011. Clinical significance of serum diamine oxidase activity in inflammatory bowel disease: Importance of evaluation of small intestinal permeability. *Inflamm. Bowel Dis.* 17:E23-E25. doi:10.1002/ibd.21588

Hopwood, D. E., D. W. Pethick, J. R. Pluske, and D. J. Hampson. 2004. Addition of pearl barley to a rice-based diet for newly weaned piglets increases the viscosity of the intestinal contents, reduces starch digestibility and exacerbates post-weaning colibacillosis. *Br. J. Nutr.* 92:419-427. doi:10.1079/BJN20041206

Hsu, J.-T., D. Faulkner, K. Garleb, R. Barclay, G. Fahey Jr, and L. Berger. 1987. Evaluation of corn fiber, cottonseed hulls, oat hulls and soybean hulls as roughage sources for ruminants. *J. Anim. Sci.* 65:244-255. doi:10.2527/jas1987.651244x

Hu, R., S. Li, H. Diao, C. Huang, J. Yan, X. Wei, M. Zhou, P. He, T. Wang, H. Fu, C. Zhong, C. Mao, Y. Wang, S. Kuang, and W. Tang. 2023. The interaction between dietary fiber and gut microbiota, and its effect on pig intestinal health. *Front. Immunol.* 14:1095740. doi:10.3389/fimmu.2023.1095740

Hu, X., J. Zhao, Q. Zhao, and J. Zheng. 2015. Structure and characteristic of β-glucan in cereal: A review. *J. Food Process. Preserv.* 39:3145-3153. doi:10.1111/jfpp.12384

Hu, Y., M. R. Aljumaah, and M. A. Azcarate-Peril. 2024. Galacto-oligosaccharides and the elderly gut: implications for immune restoration and health. *Adv. Nutr.* 15:100263. doi:10.1016/j.advnut.2024.100263

Huang, C.-S., M. E. Anderson, and A. Meister. 1993. Amino acid sequence and function of the light subunit of rat kidney gamma-glutamylcysteine synthetase. *J. Biol. Chem.* 268:20578-20583. doi:10.1016/S0021-9258(20)80764-9

Huang, G., Z. Wang, G. Wu, R. Zhang, L. Dong, F. Huang, M. Zhang, and D. Su. 2021. Lychee (*Litchi chinensis* Sonn.) pulp phenolics activate the short-chain fatty acid-free fatty acid receptor anti-inflammatory pathway by regulating microbiota and mitigate intestinal barrier damage in dextran sulfate sodium-induced colitis in mice. *J. Agric. Food. Chem.* 69:3326-3339. doi:10.1021/acs.jafc.0c07407

Huang, S., X. Rong, M. Liu, Z. Liang, Y. Geng, X. Wang, J. Zhang, C. Ji, L. Zhao, and Q. Ma. 2022. Intestinal mucosal immunity-mediated modulation of the gut microbiome by oral delivery of *Enterococcus faecium* against salmonella enteritidis

pathogenesis in a laying hen model. *Front. Immunol.* 13:853954. doi:10.3389/fimmu.2022.853954

Hussain, T., J. Wang, G. Murtaza, E. Metwally, H. Yang, M. S. Kalhoro, D. H. Kalhoro, B. A. Rahu, B. Tan, R. G. A. Sahito, M. I. Chughtai, and Y. Yin. 2021. The role of polyphenols in regulation of heat shock proteins and gut microbiota in weaning stress. *Oxid. Med. Cell. Longevity* 2021:6676444. doi:10.1155/2021/6676444

Hybertson, B. M., B. Gao, S. K. Bose, and J. M. McCord. 2011. Oxidative stress in health and disease: the therapeutic potential of Nrf2 activation. *Mol. Aspects Med.* 32:234-246. doi:10.1016/j.mam.2011.10.006

Irwin, M. R. 2023. Sleep disruption induces activation of inflammation and heightens risk for infectious disease: Role of impairments in thermoregulation and elevated ambient temperature. *Temperature* 10:198-234. doi:10.1080/23328940.2022.2109932

Jacobi, S. K., and J. Odle. 2012. Nutritional factors influencing intestinal health of the neonate. *Adv. Nutr.* 3:687-696. doi:10.3945/an.112.002683

Jandl, B., S. Dighe, C. Gasche, A. Makristathis, and M. Muttenthaler. 2024. Intestinal biofilms: pathophysiological relevance, host defense, and therapeutic opportunities. *Clin. Microbiol. Rev.* 37:e00133-00123. doi:10.1128/cmr.00133-23

Jang, K. B., Y. Kim, J. Ahn, J. I. Lee, S. Park, J. Choe, Y. Kim, J. H. Lee, H. Kyoung, and M. Song. 2024a. Dietary β-mannanase reduced post-weaning diarrhea of pigs by positively modulating gut microbiota and attenuating systemic immune responses. *Anim. Microbiome* 6:59. doi:10.1186/s42523-024-00346-z

Jang, K. B., Y. I. Kim, M. E. Duarte, and S. W. Kim. 2024b. Effects of β-mannanase supplementation on intestinal health and growth of nursery pigs. *J. Anim. Sci.* 102:skae052. doi:10.1093/jas/skae052

Janssens, S., and R. Beyaert. 2003. Role of Toll-like receptors in pathogen recognition. *Clin. Microbiol. Rev.* 16:637-646. doi:10.1128/cmr.16.4.637-646.2003

Jarrett, S., and C. J. Ashworth. 2018. The role of dietary fibre in pig production, with a particular emphasis on reproduction. *J. Anim. Sci. Biotechnol.* 9:1-11. doi:10.1186/s40104-018-0270-0

Jaworski, N. W., and H. H. Stein. 2017. Disappearance of nutrients and energy in the stomach and small intestine, cecum, and colon of pigs fed corn-soybean meal diets containing distillers dried grains with solubles, wheat middlings, or soybean hulls. *J. Anim. Sci.* 95:727-739. doi:10.2527/jas.2016.0752

Jensen, B. A. H., M. Heyndrickx, D. Jonkers, A. Mackie, S. Millet, M. Naghibi, S. I. Pærregaard, B. Pot, D. Saulnier, C. Sina, L. G. W. Sterkman, P. Van den Abbeele, N. V. Venlet, E. G. Zoetendal, and A. C. Ouwehand. 2023. Small intestine vs. colon ecology and physiology: Why it matters in probiotic administration. *Cell Rep. Med.* 4:101190. doi:10.1016/j.xcrm.2023.101190

Jensen, S. K., S. I. Pærregaard, E. P. Brandum, A. S. Jørgensen, G. M. Hjortø, and B. A. H. Jensen. 2022. Rewiring host-microbe interactions and barrier function during gastrointestinal inflammation. *Gastroenterol. Rep.* 10:goac008. doi:10.1093/gastro/goac008

Jha, R., and J. Berrocoso. 2015. Dietary fiber utilization and its effects on physiological functions and gut health of swine. *Animal* 9:1441-1452. doi:10.1017/S1751731115000919

Jha, R., J. M. Fouhse, U. P. Tiwari, L. Li, and B. P. Willing. 2019. Dietary fiber and intestinal health of monogastric animals. *Front. Vet. Sci.* 6:48. doi:10.3389/fvets.2019.00048

Jha, R., and P. Leterme. 2012. Feed ingredients differing in fermentable fibre and indigestible protein content affect fermentation metabolites and faecal nitrogen excretion in growing pigs. *Animal* 6:603-611. doi:10.1017/S1751731111001844

Jha, R., B. Rossnagel, R. Pieper, A. Van Kessel, and P. Leterme. 2010. Barley and oat cultivars with diverse carbohydrate composition alter ileal and total tract nutrient digestibility and fermentation metabolites in weaned piglets. *Animal* 4:724-731. doi:10.1017/S1751731109991510

Ji, J., S. Hu, M. Zheng, W. Du, Q. Shang, and W. Li. 2013. *Bacillus amyloliquefaciens* SC06 inhibits ETEC-induced pro-inflammatory responses by suppression of MAPK signaling pathways in IPEC-1 cells and diarrhea in weaned piglets. *Livest. Sci.* 158:206-214. doi:10.1016/j.livsci.2013.09.017

Jiménez, M. J., R. Berrios, S. Stelzhammer, M. Hohmann, W. Verri Jr, and A. P. F. Bracarense. 2020. Ingestion of organic acids and cinnamaldehyde improves tissue homeostasis of piglets exposed to enterotoxic *Escherichia coli* (ETEC). *J. Anim. Sci.* 98:skaa012. doi:0.1093/jas/skaa012

Jin, S., C. U. B. Wijerathne, K. K. W. Au-Yeung, H. Lei, C. Yang, and K. O. 2022. Effects of high- and low-fiber diets on intestinal oxidative stress in growing-finishing pigs. *J. Anim. Sci.* 100:skac306. doi:10.1093/jas/skac306

Jin, S., H. Xu, C. Yang, and K. O. 2024. Regulation of oxidative stress in the intestine of piglets after enterotoxigenic *Escherichia coli* (ETEC) infection. *Biochim. Biophys. Acta, Mol. Cell Res.* 1871:119711. doi:10.1016/j.bbamcr.2024.119711

Jo, E.-K., J. K. Kim, D.-M. Shin, and C. Sasakawa. 2016. Molecular mechanisms regulating NLRP3 inflammasome activation. *Cell. Mol. Immunol.* 13:148-159. doi:10.1038/cmi.2015.95

Johansson, E., E. Jennische, S. Lange, and I. Lönnroth. 1997. Antisecretory factor suppresses intestinal inflammation and hypersecretion. *Gut* 41:642. doi:10.1136/gut.41.5.642

Johansson, M. E. V., D. Ambort, T. Pelaseyed, A. Schütte, J. K. Gustafsson, A. Ermund, D. B. Subramani, J. M. Holmén-Larsson, K. A. Thomsson, J. H. Bergström, S. van der Post, A. M. Rodriguez-Piñeiro, H. Sjövall, M. Bäckström, and G. C. Hansson. 2011. Composition and functional role of the mucus layers in the intestine. *Cell. Mol. Life Sci.* 68:3635-3641. doi:10.1007/s00018-011-0822-3

John, L. J., M. Fromm, and J.-D. Schulzke. 2011. Epithelial barriers in intestinal inflammation. *Antioxid. Redox Signaling* 15:1255-1270. doi:10.1089/ars.2011.3892

Jørgensen, H., X.-q. Zhao, and B. O. Eggum. 1996. The influence of dietary fibre and environmental temoperature on the development of the gastrointestinal tract,

digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. *Br. J. Nutr.* 75:365-378. doi:10.1079/BJN19960140

Jung, C., J.-P. Hugot, and F. Barreau. 2010. Peyer′ s patches: The immune sensors of the intestine. *Int. J. Inflamm.* 2010:823710. doi:10.4061/2010/823710

Kanner, J. 2023. Food polyphenols as preventive medicine. *Antioxidants* 12:2103. doi:10.3390/antiox12122103

Karr-Lilienthal, L. K., C. T. Kadzere, C. M. Grieshop, and G. C. Fahey. 2005. Chemical and nutritional properties of soybean carbohydrates as related to nonruminants: A review. *Livest. Prod. Sci.* 97:1-12. doi:10.1016/j.livprodsci.2005.01.015

Keely, S. J., and K. E. Barrett. 2022. Intestinal secretory mechanisms and diarrhea. *Am. J. Physiol. Gastrointest. Liver Physiol.* 322:G405-G420. doi:10.1152/ajpgi.00316.2021

Khalil, I., J. D. Anderson, K. H. Bagamian, S. Baqar, B. Giersing, W. P. Hausdorff, C. Marshall, C. K. Porter, R. I. Walker, and A. L. Bourgeois. 2023. Vaccine value profile for enterotoxigenic *Escherichia coli* (ETEC). *Vaccine* 41:S95-S113. doi:0.1016/j.vaccine.2023.02.011

Khoshbin, K., and M. Camilleri. 2020. Effects of dietary components on intestinal permeability in health and disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 319:G589-G608. doi:10.1152/ajpgi.00245.2020

Kidd, P. M. 1997. Glutathione: systemic protectant against oxidative and free radical damage. *Altern Med Rev* 2:155-176.

Kim, C. H. 2021. Control of lymphocyte functions by gut microbiota-derived short-chain fatty acids. *Cell. Mol. Immunol.* 18:1161-1171. doi:10.1038/s41423-020-00625-0

Kim, J. C., B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2008. Addition of oat hulls to an extruded rice-based diet for weaner pigs ameliorates the incidence of diarrhoea and reduces indices of protein fermentation in the gastrointestinal tract. *Br. J. Nutr.* 99:1217-1225. doi:10.1017/S0007114507868462

Kim, K., Y. He, C. Jinno, L. Kovanda, X. Li, D. Bravo, E. Cox, and Y. Liu. 2022a. Supplementation of oligosaccharide-based polymer enhanced growth and disease resistance of weaned pigs by modulating intestinal integrity and systemic immunity. *J. Anim. Sci. Biotechnol.* 13:1-14. doi:10.1186/s40104-021-00655-2

Kim, K., M. Song, Y. Liu, and P. Ji. 2022b. Enterotoxigenic *Escherichia coli* infection of weaned pigs: Intestinal challenges and nutritional intervention to enhance disease resistance. *Front. Immunol.* 13:885253. doi:10.3389/fimmu.2022.885253

Kim, Y. J., S. B. Cho, M. H. Song, S. I. Lee, S. M. Hong, W. Yun, J. H. Lee, H. J. Oh, S. Y. Chang, J. W. An, Y. B. Go, D. C. Song, H. A. Cho, H. B. Kim, and J. H. Cho. 2022c. Effects of different *Bacillus licheniformis* and *Bacillus subtilis* ratios on nutrient digestibility, fecal microflora, and gas emissions of growing pigs. *J. Anim. Sci. Technol.* 64:291-301. doi:10.5187/jast.2022.e12

Kim, Y. J., E.-H. Kim, and K. B. Hahm. 2012. Oxidative stress in inflammation-based gastrointestinal tract diseases: Challenges and opportunities. *J. Gastroenterol. Hepatol.* 27:1004-1010. doi:10.1111/j.1440-1746.2012.07108.x

Ko, C.-W., J. Qu, D. D. Black, and P. Tso. 2020. Regulation of intestinal lipid metabolism: current concepts and relevance to disease. *Nat. Rev. Gastroenterol. Hepatol.* 17:169-183. doi:10.1038/s41575-019-0250-7

Koch, T. R., L.-X. Yuan, S. J. Stryker, P. Ratliff, G. L. Telford, and E. C. Opara. 2000. Total antioxidant capacity of colon in patients with chronic ulcerative colitis. *Dig. Dis. Sci.* 45:1814-1819. doi:10.1023/A:1005517824877

Koehler, S., A. Springer, N. Issel, S. Klinger, C. Strube, and G. Breves. 2021. Changes in porcine nutrient transport physiology in response to *Ascaris suum* infection. *Parasit Vectors* 14:533. doi:10.1186/s13071-021-05029-1

Kong, Y., K. J. Olejar, S. L. W. On, and V. Chelikani. 2020. The potential of *Lactobacillus* spp. for modulating oxidative stress in the gastrointestinal tract. *Antioxidants* 9:610. doi:10.3390/antiox9070610

Kopacz, A., D. Kloska, H. J. Forman, A. Jozkowicz, and A. Grochot-Przeczek. 2020. Beyond repression of Nrf2: An update on Keap1. *Free Radic. Biol. Med.* 157:63-74. doi:10.1016/j.freeradbiomed.2020.03.023

Kotlyarov, S., and A. Kotlyarova. 2022. Involvement of fatty acids and their metabolites in the development of inflammation in atherosclerosis. *Int. J. Mol. Sci.* 23:1308. doi:10.3390/ijms23031308

Koyyada, A. 2021. Long-term use of proton pump inhibitors as a risk factor for various adverse manifestations. *Therapies* 76:13-21. doi:10.1016/j.therap.2020.06.019

Kraehenbuhl, J.-P., and M. R. Neutra. 2000. Epithelial M cells: differentiation and function. *Annu. Rev. Cell Dev. Biol.* 16:301-332. doi:10.1146/annurev.cellbio.16.1.301

Kumar, H., R. Dhalaria, S. Guleria, R. Cimler, R. Sharma, S. A. Siddiqui, M. Valko, E. Nepovimova, D. S. Dhanjal, R. Singh, V. Kumar, A. K. Pathera, N. Verma, T. Kaur, S. Manickam, S. Y. Alomar, and K. Kuča. 2023. Anti-oxidant potential of plants

and probiotic spp. in alleviating oxidative stress induced by H_2O_2. *Biomed. Pharmacother.* 165:115022. doi:10.1016/j.biopha.2023.115022

Kunst, C., S. Schmid, M. Michalski, D. Tümen, J. Buttenschön, M. Müller, and K. Gülow. 2023. The influence of gut microbiota on oxidative stress and the immune system. *Biomedicines* 11:1388. doi:10.3390/biomedicines11051388

Kwiatkowska, E., L. Domański, V. Dziedziejko, A. Kajdy, K. Stefańska, and S. Kwiatkowski. 2021. The mechanism of drug nephrotoxicity and the methods for preventing kidney damage. *Int. J. Mol. Sci.* 22:6109. doi:10.3390/ijms22116109

Lee, C. Y., S. J. Kim, B. C. Park, and J. H. Han. 2017. Effects of dietary supplementation of bacteriophages against enterotoxigenic *Escherichia coli* (ETEC) K88 on clinical symptoms of post-weaning pigs challenged with the ETEC pathogen. *J. Anim. Physiol. Anim. Nutr.* 101:88-95. doi:10.1111/jpn.12513

Lee, H., C. Perkins, H. Gray, S. Hajat, M. Friel, R. P. Smith, S. Williamson, P. Edwards, and L. M. Collins. 2020. Influence of temperature on prevalence of health and welfare conditions in pigs: time-series analysis of pig abattoir inspection data in England and Wales. *Epidemiol. Infect.* 148:e30. doi:10.1017/S0950268819002085

Li, H., Z. Shang, X. Liu, Y. Qiao, K. Wang, and J. Qiao. 2021a. *Clostridium butyricum* alleviates enterotoxigenic *Escherichia coli* K88-induced oxidative damage through regulating the p62-Keap1-Nrf2 signaling pathway and remodeling the cecal microbial community. *Front. Immunol.* 12:771826. doi:10.3389/fimmu.2021.771826

Li, L., P. Peng, N. Ding, W. Jia, C. Huang, and Y. Tang. 2023a. Oxidative stress, inflammation, gut dysbiosis: what can polyphenols do in inflammatory bowel disease? *Antioxidants* 12:967. doi:10.3390/antiox12040967

Li, L., X. Sun, D. Zhao, and H. Dai. 2021b. Pharmacological applications and action mechanisms of phytochemicals as alternatives to antibiotics in pig production. *Front. Immunol.* 12:798553. doi:10.3389/fimmu.2021.798553

Li, Q. 2021. Structure, application, and biochemistry of microbial keratinases. *Front. Microbiol.* 12:674345. doi:10.3389/fmicb.2021.674345

Li, Q., S. Yang, X. Zhang, X. Liu, Z. Wu, Y. Qi, W. Guan, M. Ren, and S. Zhang. 2022a. Maternal nutrition during late gestation and lactation: association with immunity and the inflammatory response in the offspring. *Front. Immunol.* 12:758525. doi:10.3389/fimmu.2021.758525

Li, Q., T. Zheng, H. Ding, J. Chen, B. Li, Q. Zhang, S. Yang, S. Zhang, and W. Guan. 2023b. Exploring the benefits of probiotics in gut inflammation and diarrhea-From an antioxidant perspective. *Antioxidants* 12:1342. doi:10.3390/antiox12071342

Li, S., Y. Cai, T. Guan, Y. Zhang, K. Huang, Z. Zhang, W. Cao, and X. Guan. 2024. Quinic acid alleviates high-fat diet-induced neuroinflammation by inhibiting DR3/IKK/NF-κB signaling via gut microbial tryptophan metabolites. *Gut Microbes* 16:2374608. doi:10.1080/19490976.2024.2374608

Li, T., Y.-N. Wu, H. Wang, J.-Y. Ma, S.-S. Zhai, and J. Duan. 2020a. Dapk1 improves inflammation, oxidative stress and autophagy in LPS-induced acute lung injury via p38MAPK/NF-κB signaling pathway. *Mol. Immunol.* 120:13-22. doi:10.1016/j.molimm.2020.01.014

Li, X., F. Gou, K. Xiao, J. Zhu, Q. Lin, M. Yu, Q. Hong, and C. Hu. 2023c. Effects of DON on mitochondrial function, endoplasmic reticulum stress, and endoplasmic reticulum mitochondria contact sites in the jejunum of piglets. *J. Agric. Food. Chem.* 71:13234-13243. doi:10.1021/acs.jafc.3c03380

Li, X., C.-P. Tan, Y.-F. Liu, and Y.-J. Xu. 2020b. Interactions between food hazards and intestinal barrier: impact on foodborne diseases. *J. Agric. Food. Chem*. 68:14728-14738. doi:10.1021/acs.jafc.0c07378

Li, X., M. Wang, S. Liu, X. Chen, Y. Qiao, X. Yang, J. Yao, and S. Wu. 2022b. Paternal transgenerational nutritional epigenetic effect: A new insight into nutritional manipulation to reduce the use of antibiotics in animal feeding. *Anim. Nutr.* 11:142-151. doi:10.1016/j.aninu.2022.07.002

Li, Y., P. Wang, J. Yin, S. Jin, W. Su, J. Tian, T. Li, and K. Yao. 2020c. Effects of ornithine α-ketoglutarate on growth performance and gut microbiota in a chronic oxidative stress pig model induced by d-galactose. *Food Funct.* 11:472-482. doi:10.1039/C9FO02043H

Lian, X., M. Shi, Y. Liang, Q. Lin, and L. Zhang. 2024. The effects of unconventional feed fermentation on intestinal oxidative stress in animals. *Antioxidants* 13:305. doi:10.3390/antiox13030305

Liang, L., L. Liu, W. Zhou, C. Yang, G. Mai, H. Li, and Y. Chen. 2022. Gut microbiota-derived butyrate regulates gut mucus barrier repair by activating the macrophage/WNT/ERK signaling pathway. *Clin. Sci.* 136:291-307. doi:10.1042/CS20210778

Licastro, F., G. Candore, D. Lio, E. Porcellini, G. Colonna-Romano, C. Franceschi, and C. Caruso. 2005. Innate immunity and inflammation in ageing: a key for understanding age-related diseases. *Immun. Ageing* 2:8. doi:10.1186/1742-4933-2-8

Lima, A., and M. Fonteles. 2014. From *Escherichia coli* heat-stable enterotoxin to mammalian endogenous guanylin hormones. *Braz. J. Med. Biol. Res.* 47:179-191. doi:10.1590/1414-431X20133063

Lin, P.-Y., A. Stern, H.-H. Peng, J.-H. Chen, and H.-C. Yang. 2022. Redox and metabolic regulation of intestinal barrier function and associated disorders. *Int. J. Mol. Sci.* 23:14463. doi:10.3390/ijms232214463

Lin, Q., Q. Fu, X. Li, Y. Luo, J. Luo, D. Chen, X. Mao, B. Yu, P. Zheng, Z. Huang, J. Yu, H. Yan, and J. He. 2021. Human β-defensin 118 attenuates *Escherichia coli* K88-induced inflammation and intestinal injury in mice. *Probiotics Antimicrob. Proteins* 13:586-597. doi:10.1007/s12602-020-09725-9

Liu, D., Y. Yue, L. Ping, C. Sun, T. Zheng, Y. Cheng, G. Huo, and B. Li. 2024a. *Lactobacillus delbrueckii* subsp. *bulgaricus* 1.0207 exopolysaccharides attenuate hydrogen peroxide-induced oxidative stress damage in IPEC-J2 Cells through the Keap1/Nrf2 Pathway. *Antioxidants* 13:1150. doi:10.3390/antiox13091150

Liu, H., S. Wang, L. Yang, H. Song, and D. Zhou. 2022a. Polysaccharides from Soybean Hulls and Their Functional Activities, *Phytochemicals in Soybeans*. Boca Raton (FL): CRC Press. pp. 285-300.

Liu, L., X. Lei, X. Xiao, J. Yang, J. Li, M. Ji, W. Du, H. Tan, J. Zhu, B. Li, Z. Jin, W. Liu, J. Wu, and Z. Liu. 2020a. Epidemiological and clinical characteristics of patients

with coronavirus disease-2019 in Shiyan City, China. *Front. Cell. Infect. Microbiol*. 10:284. doi:10.3389/fcimb.2020.00284

Liu, L., C. Wu, D. Chen, B. Yu, Z. Huang, Y. Luo, P. Zheng, X. Mao, J. Yu, J. Luo, H. Yan, and J. He. 2020b. Selenium-enriched yeast alleviates oxidative stress-induced intestinal mucosa disruption in weaned pigs. *Oxid. Med. Cell. Longevity* 2020:5490743. doi:10.1155/2020/5490743

Liu, P., Y. Wang, G. Yang, Q. Zhang, L. Meng, Y. Xin, and X. Jiang. 2021. The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. *Pharmacol. Res.* 165:105420. doi:10.1016/j.phrs.2021.105420

Liu, S., J. Pi, and Q. Zhang. 2022b. Signal amplification in the KEAP1-NRF2-ARE antioxidant response pathway. *Redox Biol.* 54:102389. doi:10.1016/j.redox.2022.102389

Liu, T., W. Ma, J. Wang, Y. Wei, Y. Wang, Z. Luo, Y. Zhang, X. Zeng, W. Guan, D. Shao, and F. Chen. 2024b. Dietary protease supplementation improved growth performance and nutrients digestion via modulating intestine barrier, immunological response, and microbiota composition in weaned piglets. *Antioxidants* 13:816. doi:10.3390/antiox13070816

Liu, X., Y. Cheng, L. Shao, and Z. Ling. 2020c. Alterations of the predominant fecal microbiota and disruption of the gut mucosal barrier in patients with early-stage colorectal cancer. *Biomed Res. Int.* 2020:2948282. doi:10.1155/2020/2948282

Liu, Y., Z. Bao, X. Xu, H. Chao, C. Lin, Z. Li, Y. Liu, X. Wang, Y. You, N. Liu, and J. Ji. 2017. Extracellular signal-regulated kinase/nuclear factor-erythroid2-like2/heme

oxygenase-1 pathway-mediated mitophagy alleviates traumatic brain injury-induced intestinal mucosa damage and epithelial barrier dysfunction. *J. Neurotrauma* 34:2119-2131. doi:10.1089/neu.2016.4764

Liu, Y., N. Chen, D. Li, H. Li, Z. Fang, Y. Lin, S. Xu, B. Feng, Y. Zhuo, D. Wu, P. K. Theil, and L. Che. 2020d. Effects of dietary soluble or insoluble fiber intake in late gestation on litter performance, milk composition, immune function, and redox status of sows around parturition. *J. Anim. Sci.* 98:skaa303. doi:10.1093/jas/skaa303

Lodemann, U., S. Amasheh, J. Radloff, M. Kern, A. Bethe, L. H. Wieler, R. Pieper, J. Zentek, and J. R. Aschenbach. 2017. Effects of ex vivo infection with ETEC on jejunal barrier properties and cytokine expression in probiotic-supplemented pigs. *Dig. Dis. Sci.* 62:922-933. doi:10.1007/s10620-016-4413-x

López-Colom, P., L. Castillejos, A. Rodríguez-Sorrento, M. Puyalto, J. J. Mallo, and S. M. Martín-Orúe. 2020. Impact of in-feed sodium butyrate or sodium heptanoate protected with medium-chain fatty acids on gut health in weaned piglets challenged with *Escherichia coli* $F4^+$. *Arch. Anim. Nutr.* 74:271-295. doi:10.1080/1745039X.2020.1726719

Low, C. X., L. T.-H. Tan, N.-S. Ab Mutalib, P. Pusparajah, B.-H. Goh, K.-G. Chan, V. Letchumanan, and L.-H. Lee. 2021. Unveiling the impact of antibiotics and alternative methods for animal husbandry: a review. *Antibiotics* 10:578. doi:10.3390/antibiotics10050578

Lu, H., X. Xu, D. Fu, Y. Gu, R. Fan, H. Yi, X. He, C. Wang, B. Ouyang, P. Zhao, L. Wang, P. Xu, S. Cheng, Z. Wang, D. Zou, L. Han, and W. Zhao. 2022. Butyrate-producing

Eubacterium rectale suppresses lymphomagenesis by alleviating the TNF-induced TLR4/MyD88/NF-κB axis. *Cell Host Microbe* 30:1139-1150.e1137. doi:10.1016/j.chom.2022.07.003

Lu, S. C. 2013. Glutathione synthesis. *Biochim. Biophys. Acta, Gen. Subj.* 1830:3143-3153. doi:10.1016/j.bbagen.2012.09.008

Luise, D., P. Bosi, L. Raff, L. Amatucci, S. Virdis, and P. Trevisi. 2022. *Bacillus* spp. probiotic strains as a potential tool for limiting the use of antibiotics, and improving the growth and health of pigs and chickens. *Front. Microbiol.* 13:801827. doi:10.3389/fmicb.2022.801827

Luise, D., V. Motta, C. Boudry, C. Salvarani, F. Correa, M. Mazzoni, P. Bosi, and P. Trevisi. 2020. The supplementation of a corn/barley-based diet with bacterial xylanase did not prevent diarrhoea of ETEC susceptible piglets, but favoured the persistence of Lactobacillus reuteri in the gut. *Livest. Sci.* 240:104161. doi:10.1016/j.livsci.2020.104161

Luissint, A.-C., C. A. Parkos, and A. Nusrat. 2016. Inflammation and the intestinal barrier: leukocyte-epithelial cell interactions, cell junction remodeling, and mucosal repair. *Gastroenterology* 151:616-632. doi:10.1053/j.gastro.2016.07.008

Luk, G. D., T. M. Bayless, and S. B. Baylin. 1980. Diamine oxidase (histaminase). A circulating marker for rat intestinal mucosal maturation and integrity. *J. Clin. Investig.* 66:66-70. doi:10.1172/JCI109836

Luo, Y., L. Liu, D. Chen, B. Yu, P. Zheng, X. Mao, Z. Huang, J. Yu, J. Luo, H. Yan, and J. He. 2022a. Dietary supplementation of fructo-oligosaccharides alleviates

enterotoxigenic *E. coli*-induced disruption of intestinal epithelium in a weaned piglet model. *Br. J. Nutr.* 128:1526-1534. doi:10.1017/S0007114521004451

Luo, Y., W. Ren, H. Smidt, G. Wright André-Denis, B. Yu, G. Schyns, M. McCormack Ursula, J. Cowieson Aaron, J. Yu, J. He, H. Yan, J. Wu, I. Mackie Roderick, and D. Chen. 2022b. Dynamic distribution of gut microbiota in pigs at different growth stages: composition and contribution. *Microbiol. Spectrum* 10:e00688-00621. doi:10.1128/spectrum.00688-21

Lupberger, J., K.-A. Kreuzer, G. Baskaynak, U. Peters, P. Le Coutre, and C. Schmidt. 2002. Quantitative analysis of beta-actin, beta-2-microglobulin and porphobilinogen deaminase mRNA and their comparison as control transcripts for RT-PCR. *Mol. Cell. Probes.* 16:25-30. doi:10.1006/mcpr.2001.0392

Ma, K., B. Su, F. Li, J. Li, J. Nie, W. Xiong, J. Luo, S. Huang, T. Zhou, X. Liang, F. Li, J. Deng, and C. Tan. 2024. Maternal or post-weaning dietary fructo-oligosaccharide supplementation reduces stillbirth rate of sows and diarrhea of weaned piglets. *Anim. Nutr.* 17:155-164. doi:10.1016/j.aninu.2024.04.002

Ma, L., and L. Morel. 2022. Loss of gut barrier integrity in lupus. *Front. Immunol.* 13:919792. doi:10.3389/fimmu.2022.919792

Ma, Y., X. Han, J. Fang, and H. Jiang. 2022. Role of dietary amino acids and microbial metabolites in the regulation of pig intestinal health. *Anim. Nutr.* 9:1-6. doi:10.1016/j.aninu.2021.10.004

Mackie, A., A.-I. Mulet-Cabero, and A. Torcello-Gómez. 2020. Simulating human digestion: developing our knowledge to create healthier and more sustainable foods. *Food Funct.* 11:9397-9431. doi:10.1039/D0FO01981J

Manzoor, S., S. M. Wani, S. Ahmad Mir, and D. Rizwan. 2022. Role of probiotics and prebiotics in mitigation of different diseases. *Nutrition* 96:111602. doi:10.1016/j.nut.2022.111602

Mao, J., S. Qi, Y. Cui, X. Dou, X. M. Luo, J. Liu, T. Zhu, Y. Ma, and H. Wang. 2020. *Lactobacillus rhamnosus* GG attenuates lipopolysaccharide-induced inflammation and barrier dysfunction by regulating MAPK/NF-κB signaling and modulating metabolome in the piglet intestine. *J. Nutr.* 150:1313-1323. doi:10.1093/jn/nxaa009

Marí, M., A. Morales, A. Colell, C. García-Ruiz, and J. C. Fernández-Checa. 2009. Mitochondrial glutathione, a key survival antioxidant. *Antioxid. Redox Signaling* 11:2685-2700. doi:10.1089/ars.2009.2695

Markowiak-Kopeć, P., and K. Śliżewska. 2020. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients* 12:1107. doi:10.3390/nu12041107

Martel, J., S.-H. Chang, Y.-F. Ko, T.-L. Hwang, J. D. Young, and D. M. Ojcius. 2022. Gut barrier disruption and chronic disease. *Trends Endocrinol. Metab.* 33:247-265. doi:10.1016/j.tem.2022.01.002

Martemucci, G., C. Costagliola, M. Mariano, L. D'andrea, P. Napolitano, and A. G. D'Alessandro. 2022. Free radical properties, source and targets, antioxidant consumption and health. *Oxygen* 2:48-78. doi:10.3390/oxygen2020006

Martini, H., and J. F. Passos. 2023. Cellular senescence: all roads lead to mitochondria. *FEBS J.* 290:1186-1202. doi:10.1111/febs.16361

Mavrommatis, A., E. Giamouri, S. Tavrizelou, M. Zacharioudaki, G. Danezis, P. E. Simitzis, E. Zoidis, E. Tsiplakou, A. C. Pappas, C. A. Georgiou, and K. Feggeros. 2021. Impact of mycotoxins on animals' oxidative status. *Antioxidants* 10:214. doi:10.3390/antiox10020214

Mavrommatis, A., E. Tsiplakou, A. Zerva, P. D. Pantiora, N. D. Georgakis, G. P. Tsintzou, P. Madesis, and N. E. Labrou. 2023. Microalgae as a sustainable source of antioxidants in animal nutrition, health and livestock development. *Antioxidants* 12:1882. doi:10.3390/antiox12101882

Mazumder, S., S. Bindu, R. De, S. Debsharma, S. Pramanik, and U. Bandyopadhyay. 2022. Emerging role of mitochondrial DAMPs, aberrant mitochondrial dynamics and anomalous mitophagy in gut mucosal pathogenesis. *Life Sci.* 305:120753. doi:10.1016/j.lfs.2022.120753

McFadden, D., M. J. Zinner, and B. M. Jaffe. 1986. Substance P-induced intestinal secretion of water and electrolytes. *Gut* 27:267. doi:10.1136/gut.27.3.267

McKEE, L. H., and T. Latner. 2000. Underutilized sources of dietary fiber: A review. *Plant Foods Hum. Nutr.* 55:285-304. doi:10.1023/A:1008144310986

Megur, A., E. B.-M. Daliri, D. Baltriukienė, and A. Burokas. 2022. Prebiotics as a tool for the prevention and treatment of obesity and diabetes: classification and ability to modulate the gut microbiota. *Int. J. Mol. Sci.* 23:6097. doi:10.3390/ijms23116097

Meijnikman, A. S., M. Nieuwdorp, and B. Schnabl. 2024. Endogenous ethanol production in health and disease. *Nat. Rev. Gastroenterol. Hepatol.* 21:556-571. doi:10.1038/s41575-024-00937-w

Miner-Williams, W. M., and P. J. Moughan. 2016. Intestinal barrier dysfunction: implications for chronic inflammatory conditions of the bowel. *Nutr. Res. Rev.* 29:40-59. doi:10.1017/S0954422416000019

Mirończuk-Chodakowska, I., A. M. Witkowska, and M. E. Zujko. 2018. Endogenous non-enzymatic antioxidants in the human body. *Adv. Med. Sci.* 63:68-78. doi:10.1016/j.advms.2017.05.005

Mo, J., J. Ni, M. Zhang, Y. Xu, Y. Li, N. Karim, and W. Chen. 2022. Mulberry anthocyanins ameliorate DSS-induced ulcerative colitis by improving intestinal barrier function and modulating gut microbiota. *Antioxidants* 11:1674. doi:10.3390/antiox11091674

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

Mohnen, D. 2008. Pectin structure and biosynthesis. *Curr. Opin. Plant Biol.* 11:266-277. doi:10.1016/j.pbi.2008.03.006

Monger, X. C., A.-A. Gilbert, L. Saucier, and A. T. Vincent. 2021. Antibiotic resistance: from pig to meat. *Antibiotics* 10:1209. doi:10.3390/antibiotics10101209

Montagne, L., J. Pluske, and D. Hampson. 2003. A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. *Anim. Feed Sci. Technol.* 108:95-117. doi:10.1016/S0377-8401(03)00163-9

Mostafavi Abdolmaleky, H., and J.-R. Zhou. 2024. Gut microbiota dysbiosis, oxidative stress, inflammation, and epigenetic alterations in metabolic diseases. *Antioxidants* 13:985. doi:10.3390/antiox13080985

Motta, J.-P., J. L. Wallace, A. G. Buret, C. Deraison, and N. Vergnolle. 2021. Gastrointestinal biofilms in health and disease. *Nat. Rev. Gastroenterol. Hepatol.* 18:314-334. doi:10.1038/s41575-020-00397-y

Mousavi Khaneghah, A., K. Abhari, I. Eş, M. B. Soares, R. B. A. Oliveira, H. Hosseini, M. Rezaei, C. F. Balthazar, R. Silva, A. G. Cruz, C. S. Ranadheera, and A. S. Sant'Ana. 2020. Interactions between probiotics and pathogenic microorganisms in hosts and foods: A review. *Trends Food Sci. Technol.* 95:205-218. doi:10.1016/j.tifs.2019.11.022

Mowat, A. M., and W. W. Agace. 2014. Regional specialization within the intestinal immune system. *Nat. Rev. Immunol.* 14:667-685. doi:10.1038/nri3738

Mukherjee, T., N. Kumar, M. Chawla, D. J. Philpott, and S. Basak. 2024. The NF-κB signaling system in the immunopathogenesis of inflammatory bowel disease. *Sci. Signaling* 17:eadh1641. doi:10.1126/scisignal.adh1641

Munteanu, C., and B. Schwartz. 2024. Interactions between dietary antioxidants, dietary fiber and the gut microbiome: their putative role in inflammation and Cancer. *Int. J. Mol. Sci.* 25:8250. doi:10.3390/ijms25158250

Muramatsu, M. K., and S. E. Winter. 2024. Nutrient acquisition strategies by gut microbes. *Cell Host Microbe* 32:863-874. doi:10.1016/j.chom.2024.05.011

Muscolo, A., O. Mariateresa, T. Giulio, and R. Mariateresa. 2024. Oxidative stress: the role of antioxidant phytochemicals in the prevention and treatment of diseases. *Int. J. Mol. Sci.* 25:3264. doi:10.3390/ijms25063264

Muteeb, G., M. T. Rehman, M. Shahwan, and M. Aatif. 2023. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals* 16:1615. doi:10.3390/ph16111615

Muthyala, S. D. V., S. Shankar, C. Klemashevich, J. C. Blazier, A. Hillhouse, and C.-S. Wu. 2022. Differential effects of the soluble fiber inulin in reducing adiposity and altering gut microbiome in aging mice. *J. Nutr. Biochem.* 105:108999. doi:10.1016/j.jnutbio.2022.108999

Muwonge, A., A. K. Karuppannan, and T. Opriessnig. 2021. Probiotics mediated gut microbiota diversity shifts are associated with reduction in histopathology and shedding of Lawsonia intracellularis. *Anim. Microbiome* 3:22. doi:10.1186/s42523-021-00084-6

Nassour, H., and J. D. Dubreuil. 2014. *Escherichia coli* STb enterotoxin dislodges claudin-1 from epithelial tight junctions. *PLoS One* 9:e113273. doi:10.1371/journal.pone.0113273

Ndou, S., E. Kiarie, A. Agyekum, J. Heo, L. Romero, S. Arent, R. Lorentsen, and C. Nyachoti. 2015. Comparative efficacy of xylanases on growth performance and digestibility in growing pigs fed wheat and wheat bran-or corn and corn DDGS-based diets supplemented with phytase. *Anim. Feed Sci. Technol.* 209:230-239. doi:10.1016/j.anifeedsci.2015.08.011

Ndou, S., E. Kiarie, S. Thandapilly, M. Walsh, N. Ames, and C. Nyachoti. 2017. Flaxseed meal and oat hulls supplementation modulates growth performance, blood lipids, intestinal fermentation, bile acids, and neutral sterols in growing pigs fed corn–soybean meal–based diets. *J. Anim. Sci.* 95:3068-3078. doi:10.2527/jas.2016.1328

Neurath, M. F. 2014. Cytokines in inflammatory bowel disease. *Nat. Rev. Immunol.* 14:329-342. doi:10.1038/nri3661

Ngendahayo Mukiza, C., and J. D. Dubreuil. 2013. *Escherichia coli* heat-stable toxin b impairs intestinal epithelial barrier function by altering tight junction proteins. *Infect. Immun.* 81:2819-2827. doi:10.1128/iai.00455-13

Nielsen, C., F. V. Mortensen, E. J. Erlandsen, and J. S. Lindholt. 2012. L- and D-lactate as biomarkers of arterial-induced intestinal ischemia: An experimental study in pigs. *Int. Surg. J.* 10:296-300. doi:10.1016/j.ijsu.2012.05.003

Noel, G., M. Doucet, J. P. Nataro, J. B. Kaper, N. C. Zachos, and M. F. Pasetti. 2018. Enterotoxigenic Escherichia coli is phagocytosed by macrophages underlying villus-like intestinal epithelial cells: modeling ex vivo innate immune defenses of the human gut. *Gut Microbes* 9:382-389. doi:10.1080/19490976.2017.1398871

Nogal, A., A. M. Valdes, and C. Menni. 2021. The role of short-chain fatty acids in the interplay between gut microbiota and diet in cardio-metabolic health. *Gut Microbes* 13:1897212. doi:10.1080/19490976.2021.1897212

Nolfi-Donegan, D., A. Braganza, and S. Shiva. 2020. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. *Redox Biol.* 37:101674. doi:10.1016/j.redox.2020.101674

NRC. 2012. *Nutrient requirements of swine.*11th rev. ed. Washington (DC): Natl. Acad. Press.

Okoye, C. N., S. A. Koren, and A. P. Wojtovich. 2023. Mitochondrial complex I ROS production and redox signaling in hypoxia. *Redox Biol.* 67:102926. doi:10.1016/j.redox.2023.102926

Omenetti, S., and T. T. Pizarro. 2015. The Treg/Th17 axis: a dynamic balance regulated by the gut microbiome. *Front. Immunol.* 6:639. doi:10.3389/fimmu.2015.00639

Ong, G., and S. E. Logue. 2023. Unfolding the interactions between endoplasmic reticulum stress and oxidative stress. *Antioxidants* 12:981. doi:10.3390/antiox12050981

Oroian, B. A., A. Ciobica, D. Timofte, C. Stefanescu, and I. L. Serban. 2021. New metabolic, digestive, and oxidative stress-related manifestations associated with posttraumatic stress disorder. *Oxid. Med. Cell. Longevity* 2021:5599265. doi:10.1155/2021/5599265

Palkovicsné Pézsa, N., D. Kovács, B. Rácz, and O. Farkas. 2022. Effects of *Bacillus licheniformis* and *Bacillus subtilis* on gut barrier function, proinflammatory response, ROS production and pathogen inhibition properties in IPEC-J2-*Escherichia coli*/*Salmonella Typhimurium* co-culture. *Microorganisms* 10:936. doi:10.3390/microorganisms10050936

Panwar, S., S. Sharma, and P. Tripathi. 2021. Role of barrier integrity and dysfunctions in maintaining the healthy gut and their health outcomes. *Front. Physiol.* 12:715611. doi:10.3389/fphys.2021.715611

Paone, P., and P. D. Cani. 2020. Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut* 69:2232. doi:10.1136/gutjnl-2020-322260

Paradis, T., H. Bègue, L. Basmaciyan, F. Dalle, and F. Bon. 2021. Tight junctions as a key for pathogens invasion in intestinal epithelial cells. *Int. J. Mol. Sci.* 22:2506. doi:10.3390/ijms22052506

Patel, S., and B. A. McCormick. 2014. Mucosal Inflammatory Response to *Salmonella typhimurium* Infection. *Front. Immunol.* 5:311. doi:10.3389/fimmu.2014.00311

Perdue, M. H., and D. M. McKay. 1994. Integrative immunophysiology in the intestinal mucosa. *Am. J. Physiol. Gastrointest. Liver Physiol.* 267:G151-G165. doi:10.1152/ajpgi.1994.267.2.G151

Pereira, P. M. d. C. C., and A. F. d. R. B. Vicente. 2013. Meat nutritional composition and nutritive role in the human diet. *Meat Sci.* 93:586-592. doi:10.1016/j.meatsci.2012.09.018

Peterson, L. W., and D. Artis. 2014. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat. Rev. Immunol.* 14:141-153. doi:10.1038/nri3608

Petrova, P., A. Arsov, I. Ivanov, L. Tsigoriyna, and K. Petrov. 2021. New exopolysaccharides produced by *Bacillus licheniformis* 24 display substrate-dependent content and antioxidant activity. *Microorganisms* 9:2127. doi:10.3390/microorganisms9102127

Phillips, S. F. 1969. Absorption and secretion by the colon. *Gastroenterology* 56:966-971. doi:10.1016/S0016-5085(69)80101-0

Pickard, J. M., M. Y. Zeng, R. Caruso, and G. Núñez. 2017. Gut microbiota: role in pathogen colonization, immune responses, and inflammatory disease. *Immunol. Rev.* 279:70-89. doi:10.1111/imr.12567

Pidwill, G. R., J. F. Gibson, J. Cole, S. A. Renshaw, and S. J. Foster. 2021. The role of macrophages in *Staphylococcus aureus* infection. *Front. Immunol.* 11:620339. doi:10.3389/fimmu.2020.620339

Pieper, R., R. Jha, B. Rossnagel, A. G. Van Kessel, W. B. Souffrant, and P. Leterme. 2008. Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. *FEMS Microbiol. Ecol.* 66:556-566. doi:10.1111/j.1574-6941.2008.00605.x

Pietrzak, B., K. Tomela, A. Olejnik-Schmidt, A. Mackiewicz, and M. Schmidt. 2020. Secretory IgA in intestinal mucosal secretions as an adaptive barrier against microbial cells. *Int. J. Mol. Sci.* 21:9254. doi:10.3390/ijms21239254

Plaza-Diaz, J., F. J. Ruiz-Ojeda, M. Gil-Campos, and A. Gil. 2019. Mechanisms of action of probiotics. *Adv. Nutr.* 10:S49-S66. doi:10.1093/advances/nmy063

Pluske, J. R. 2016. Invited review: Aspects of gastrointestinal tract growth and maturation in the pre- and postweaning period of pigs. *J. Anim. Sci.* 94:399-411. doi:10.2527/jas.2015-9767

Pokharel, P., S. Dhakal, and C. M. Dozois. 2023. The diversity of *Escherichia coli* pathotypes and vaccination strategies against this versatile bacterial pathogen. *Microorganisms* 11:344. doi:10.3390/microorganisms11020344

Prathapasinghe, G. A., Y. L. Siow, and K. O. 2007. Detrimental role of homocysteine in renal ischemia-reperfusion injury. *Am. J. Physiol. Renal Physiol.* 292:F1354-F1363. doi:10.1152/ajprenal.00301.2006

Priya Dharshini, L. C., S. Vishnupriya, K. M. Sakthivel, and R. R. Rasmi. 2020. Oxidative stress responsive transcription factors in cellular signalling transduction mechanisms. *Cell. Signalling* 72:109670. doi:10.1016/j.cellsig.2020.109670

Pupa, P., P. Apiwatsiri, W. Sirichokchatchawan, N. Pirarat, T. Nedumpun, D. J. Hampson, N. Muangsin, and N. Prapasarakul. 2022. Microencapsulated probiotic *Lactiplantibacillus plantarum* and/or *Pediococcus acidilactici* strains ameliorate diarrhoea in piglets challenged with enterotoxigenic *Escherichia coli*. *Sci. Rep.* 12:7210. doi:10.1038/s41598-022-11340-3

Puppel, K., A. Kapusta, and B. Kuczyńska. 2015. The etiology of oxidative stress in the various species of animals, a review. *J. Sci. Food Agric.* 95:2179-2184. doi:10.1002/jsfa.7015

Pyrillou, K., L. C. Burzynski, and M. C. H. Clarke. 2020. Alternative pathways of IL-1 activation, and its role in health and disease. *Front. Immunol.* 11:613170. doi:10.3389/fimmu.2020.613170

Qiao, L., X. Dou, X. Song, J. Chang, X. Zeng, L. Zhu, H. Yi, and C. Xu. 2023. Replacing dietary sodium selenite with biogenic selenium nanoparticles improves the growth performance and gut health of early-weaned piglets. *Anim. Nutr.* 15:99-113. doi:10.1016/j.aninu.2023.08.003

Qin, S., X. Xiao, Z. Dai, G. Zhao, Z. Cui, Y. Wu, and C. Yang. 2024a. Effects of *Bacillus licheniformis* on growth performance, immune and antioxidant functions, and intestinal microbiota of broilers. *Poult. Sci.* 103:103210. doi:10.1016/j.psj.2023.103210

Qin, W., L. Sun, M. Miao, and G. Zhang. 2021. Plant-sourced intrinsic dietary fiber: Physical structure and health function. *Trends Food Sci. Technol.* 118:341-355. doi:10.1016/j.tifs.2021.09.022

Qin, Y.-C., C.-L. Jin, T.-C. Hu, J.-Y. Zhou, X.-F. Wang, X.-Q. Wang, X.-F. Kong, and H.-C. Yan. 2024b. Early weaning inhibits intestinal stem cell expansion to disrupt the intestinal integrity of duroc piglets via regulating the Keap1/Nrf2 signaling. *Antioxidants* 13:1188. doi:10.3390/antiox13101188

Qiu, Y., J. Yang, L. Wang, X. Yang, K. Gao, C. Zhu, and Z. Jiang. 2021. Dietary resveratrol attenuation of intestinal inflammation and oxidative damage is linked to the alteration of gut microbiota and butyrate in piglets challenged with deoxynivalenol. *J. Anim. Sci. Biotechnol.* 12:71. doi:10.1186/s40104-021-00596-w

Quintana-Hayashi Macarena, P., N. Navabi, M. Mahu, V. Venkatakrishnan, R. Fernandez Harvey, F. Haesebrouck, F. Pasmans, and K. Lindén Sara. 2017. Neutrophil elastase and interleukin 17 expressed in the pig colon during *Brachyspira hyodysenteriae* infection synergistically with the pathogen induce increased mucus transport speed and production via mitogen-activated protein kinase 3. *Infect. Immun.* 85:00262-00217. doi:10.1128/iai.00262-17

Raghunath, A., K. Sundarraj, R. Nagarajan, F. Arfuso, J. Bian, A. P. Kumar, G. Sethi, and E. Perumal. 2018. Antioxidant response elements: Discovery, classes, regulation and potential applications. *Redox Biol.* 17:297-314. doi:10.1016/j.redox.2018.05.002

Raheem, A., L. Liang, G. Zhang, and S. Cui. 2021. Modulatory effects of probiotics during pathogenic infections with emphasis on immune regulation. *Front. Immunol.* 12:616713. doi:10.3389/fimmu.2021.616713

Ramamurthy, T., R. K. Nandy, A. K. Mukhopadhyay, S. Dutta, A. Mutreja, K. Okamoto, S.-I. Miyoshi, G. B. Nair, and A. Ghosh. 2020. Virulence regulation and innate host response in the pathogenicity of *Vibrio cholerae*. *Front. Cell. Infect. Microbiol*. 10:572096. doi:10.3389/fcimb.2020.572096

Ramanan, D., and K. Cadwell. 2016. Intrinsic defense mechanisms of the intestinal epithelium. *Cell Host Microbe* 19:434-441. doi:10.1016/j.chom.2016.03.003

Ramirez-Olea, H., B. Reyes-Ballesteros, and R. A. Chavez-Santoscoy. 2022. Potential application of the probiotic *Bacillus licheniformis* as an adjuvant in the treatment of diseases in humans and animals: A systematic review. *Front. Microbiol*. 13:993451. doi:10.3389/fmicb.2022.993451

Rastogi, S., and A. Singh. 2022. Gut microbiome and human health: Exploring how the probiotic genus *Lactobacillus* modulate immune responses. *Front. Pharmacol.* 13:1042189. doi:10.3389/fphar.2022.1042189

Rath, E., and D. Haller. 2022. Intestinal epithelial cell metabolism at the interface of microbial dysbiosis and tissue injury. *Mucosal Immunol.* 15:595-604. doi:10.1038/s41385-022-00514-x

Raut, S. K., and M. Khullar. 2023. Oxidative stress in metabolic diseases: current scenario and therapeutic relevance. *Mol. Cell. Biochem*. 478:185-196. doi:10.1007/s11010-022-04496-z

Rauw, W. M., L. Rydhmer, I. Kyriazakis, M. Øverland, H. Gilbert, J. C. Dekkers, S. Hermesch, A. Bouquet, E. Gómez Izquierdo, and I. Louveau. 2020. Prospects for sustainability of pig production in relation to climate change and novel feed resources. *J. Sci. Food Agric.* 100:3575-3586. doi:10.1002/jsfa.10338

Régnier, M., M. Van Hul, C. Knauf, and P. D. Cani. 2021. Gut microbiome, endocrine control of gut barrier function and metabolic diseases. *J. Endocrinol.* 248:R67-R82. doi:10.1530/JOE-20-0473

Ren, S., C. Wang, A. Chen, W. Lv, and R. Gao. 2022. The probiotic *Lactobacillus paracasei* ameliorates diarrhea cause by *Escherichia coli* O8 via gut microbiota modulation. *Front. Nutr.* 9:878808. doi:10.3389/fnut.2022.878808

Renaudeau, D., G. Frances, S. Dubois, H. Gilbert, and J. Noblet. 2013. Effect of thermal heat stress on energy utilization in two lines of pigs divergently selected for residual feed intake1,2. *J. Anim. Sci.* 91:1162-1175. doi:10.2527/jas.2012-5689

Rezende, E. S. V., G. C. Lima, and M. M. V. Naves. 2021. Dietary fibers as beneficial microbiota modulators: A proposed classification by prebiotic categories. *Nutrition* 89:111217. doi:10.1016/j.nut.2021.111217

Riaz Rajoka, M. S., R. Thirumdas, H. M. Mehwish, M. Umair, M. Khurshid, H. F. Hayat, Y. Phimolsiripol, N. Pallarés, F. J. Martí-Quijal, and F. J. Barba. 2021. Role of food antioxidants in modulating gut microbial communities: Novel understandings in intestinal oxidative stress damage and their impact on host health. *Antioxidants* 10:1563. doi:10.3390/antiox10101563

Rodrigues, L. A., B. Koo, M. Nyachoti, and D. A. Columbus. 2022. Formulating diets for improved health status of pigs: current knowledge and perspectives. *Animals* 12:2877. doi:10.3390/ani12202877

Rogers, A. P., S. J. Mileto, and D. Lyras. 2023. Impact of enteric bacterial infections at and beyond the epithelial barrier. *Nat. Rev. Microbiol.* 21:260-274. doi:10.1038/s41579-022-00794-x

Rohr, M. W., C. A. Narasimhulu, T. A. Rudeski-Rohr, and S. Parthasarathy. 2020. Negative effects of a high-fat diet on intestinal permeability: a review. *Adv. Nutr.* 11:77-91. doi:10.1093/advances/nmz061

Ruhr, I. M., C. Bodinier, E. M. Mager, A. J. Esbaugh, C. Williams, Y. Takei, and M. Grosell. 2014. Guanylin peptides regulate electrolyte and fluid transport in the Gulf toadfish (*Opsanus beta*) posterior intestine. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 307:R1167-R1179. doi:10.1152/ajpregu.00188.2014

Rummel, N. G., and D. A. Butterfield. 2021. Altered metabolism in Alzheimer disease brain: role of oxidative stress. *Antioxid. Redox Signaling* 36:1289-1305. doi:10.1089/ars.2021.0177

Russo, I., A. Luciani, P. De Cicco, E. Troncone, and C. Ciacci. 2012. Butyrate attenuates lipopolysaccharide-induced inflammation in intestinal cells and Crohn's mucosa through modulation of antioxidant defense machinery. *PLoS One* 7:e32841. doi:10.1371/journal.pone.0032841

Ruth, M. R., and C. J. Field. 2013. The immune modifying effects of amino acids on gut-associated lymphoid tissue. *J. Anim. Sci. Biotechnol.* 4:1-10. doi:10.1186/2049-1891-4-27

Sadowska-Bartosz, I., and G. Bartosz. 2023. Peroxiredoxin 2: an important element of the antioxidant defense of the erythrocyte. *Antioxidants* 12:1012. doi:10.3390/antiox12051012

Sáez-Lara, M. J., C. Robles-Sanchez, F. J. Ruiz-Ojeda, J. Plaza-Diaz, and A. Gil. 2016. Effects of probiotics and synbiotics on obesity, insulin resistance syndrome, type 2 diabetes and non-alcoholic fatty liver disease: a review of human clinical trialss. *Int. J. Mol. Sci.* 17:928. doi:10.3390/ijms17060928

Salazar-Bermeo, J., B. Moreno-Chamba, M. C. Martínez-Madrid, D. Saura, M. Valero, and N. Martí. 2021. Potential of persimmon dietary fiber obtained from byproducts as antioxidant, prebiotic and modulating agent of the intestinal epithelial barrier function. *Antioxidants* 10:1668. doi:10.3390/antiox10111668

Sánchez de Medina, F., I. Romero-Calvo, C. Mascaraque, and O. Martínez-Augustin. 2014. Intestinal inflammation and mucosal barrier function. *Inflamm. Bowel Dis.* 20:2394-2404. doi:10.1097/MIB.0000000000000204

Sarowska, J., B. Futoma-Koloch, A. Jama-Kmiecik, M. Frej-Madrzak, M. Ksiazczyk, G. Bugla-Ploskonska, and I. Choroszy-Krol. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. *Gut Pathog.* 11:10. doi:10.1186/s13099-019-0290-0

Sasson, A. N., R. J. M. Ingram, Z. Zhang, L. M. Taylor, A. N. Ananthakrishnan, G. G. Kaplan, S. C. Ng, S. Ghosh, and M. Raman. 2021. The role of precision nutrition in the modulation of microbial composition and function in people with

inflammatory bowel disease. *Lancet Gastroenterol. Hepatol.* 6:754-769. doi:10.1016/S2468-1253(21)00097-2

Schwärzler, J., L. Mayr, F. Grabherr, H. Tilg, and T. E. Adolph. 2024. Epithelial metabolism as a rheostat for intestinal inflammation and malignancy. *Trends Cell Biol.* 34:913-927. doi:10.1016/j.tcb.2024.01.004

Seelig, G. F., R. Simondsen, and A. Meister. 1984. Reversible dissociation of gamma-glutamylcysteine synthetase into two subunits. *J. Biol. Chem.* 259:9345-9347. doi:10.1016/S0021-9258(17)42703-7

Serrano-Jara, D., J. Rivera-Gomis, J. A. Tornel, M. J. Jordán, C. Martínez-Conesa, and M. J. Pablo. 2024. Oregano essential oil and purple garlic powder effects on intestinal health, microbiota indicators and antimicrobial resistance as feed additives in weaning piglets. *Animals* 14:111. doi:10.3390/ani14010111

Shah, B. R., B. Li, H. Al Sabbah, W. Xu, and J. Mráz. 2020. Effects of prebiotic dietary fibers and probiotics on human health: With special focus on recent advancement in their encapsulated formulations. *Trends Food Sci. Technol.* 102:178-192. doi:10.1016/j.tifs.2020.06.010

Shao, T., H. K. Verma, B. Pande, V. Costanzo, W. Ye, Y. Cai, and L. V. K. S. Bhaskar. 2021. Physical activity and nutritional influence on immune function: an important strategy to improve immunity and health status. *Front. Physiol.* 12:751374. doi:10.3389/fphys.2021.751374

Sharma, L., and A. Riva. 2020. Intestinal barrier function in health and disease-any role of SARS-CoV-2? *Microorganisms* 8:1744. doi:10.3390/microorganisms8111744

Sheikh, A., B. Tumala, T. J. Vickers, J. C. Martin, B. A. Rosa, S. Sabui, S. Basu, R. D. Simoes, M. Mitreva, C. Storer, E. Tyksen, R. D. Head, W. Beatty, H. M. Said, and J. M. Fleckenstein. 2022. Enterotoxigenic *Escherichia coli* heat-labile toxin drives enteropathic changes in small intestinal epithelia. *Nat. Commun.* 13:6886. doi:10.1038/s41467-022-34687-7

Shi, C.-w., M.-y. Cheng, X. Yang, Y.-y. Lu, H.-d. Yin, Y. Zeng, R.-y. Wang, Y.-l. Jiang, W.-t. Yang, J.-z. Wang, D.-d. Zhao, H.-b. Huang, L.-p. Ye, X. Cao, G.-l. Yang, and C.-f. Wang. 2020. Probiotic *Lactobacillus rhamnosus* GG promotes mouse gut microbiota diversity and T cell differentiation. *Front. Microbiol.* 11:607735. doi:10.3389/fmicb.2020.607735

Shi, S., Y. Chen, Z. Luo, G. Nie, and Y. Dai. 2023. Role of oxidative stress and inflammation-related signaling pathways in doxorubicin-induced cardiomyopathy. *Cell. Commun. Signal.* 21:61. doi:10.1186/s12964-023-01077-5

Shi, S., B. Cheng, B. Gu, T. Sheng, J. Tu, Y. Shao, K. Qi, and D. Zhou. 2021. Evaluation of the probiotic and functional potential of *Lactobacillus agilis* 32 isolated from pig manure. *Lett. Appl. Microbiol.* 73:9-19. doi:10.1111/lam.13422

Shi, Z., T. Wang, J. Kang, Y. Li, Y. Li, and L. Xi. 2022. Effects of weaning modes on the intestinal ph, activity of digestive enzymes, and intestinal morphology of piglets. *Animals* 12:2200. doi:10.3390/ani12172200

Shimura, T. 2023. Mitochondrial signaling pathways associated with DNA damage responses. *Int. J. Mol. Sci.* 24:6128. doi:10.3390/ijms24076128

Siddiqui, M. T., and G. A. M. Cresci. 2021. The immunomodulatory functions of butyrate. *J. Inflamm. Res.* 14:6025-6041. doi:10.2147/JIR.S300989

Sies, H., V. V. Belousov, N. S. Chandel, M. J. Davies, D. P. Jones, G. E. Mann, M. P. Murphy, M. Yamamoto, and C. Winterbourn. 2022. Defining roles of specific reactive oxygen species (ROS) in cell biology and physiology. *Nat. Rev. Mol. Cell Biol.* 23:499-515. doi:10.1038/s41580-022-00456-z

Sies, H., and D. P. Jones. 2020. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* 21:363-383. doi:10.1038/s41580-020-0230-3

Silva Júnior, C. D., C. C. S. Martins, F. T. F. Dias, N. Y. Sitanaka, L. B. Ferracioli, J. E. Moraes, C. C. Pizzolante, F. E. L. Budiño, R. Pereira, P. Tizioto, V. R. C. Paula, L. L. Coutinho, and U. S. Ruiz. 2020. The use of an alternative feed additive, containing benzoic acid, thymol, eugenol, and piperine, improved growth performance, nutrient and energy digestibility, and gut health in weaned piglets. *J. Anim. Sci.* 98:skaa119. doi:10.1093/jas/skaa119

Small, C.-L. N., S. A. Reid-Yu, J. B. McPhee, and B. K. Coombes. 2013. Persistent infection with Crohn's disease-associated adherent-invasive *Escherichia coli* leads to chronic inflammation and intestinal fibrosis. *Nat. Commun.* 4:1957. doi:10.1038/ncomms2957

Sobotka, W., M. Flis, Z. Antoszkiewicz, K. Lipiński, and Z. Zduńczyk. 2012. Effect of oat by-product antioxidants and vitamin E on the oxidative stability of pork from pigs fed diets supplemented with linseed oil. *Arch. Anim. Nutr.* 66:27-38. doi:10.1080/1745039X.2011.647459

Soliani, L., G. Rugna, A. Prosperi, C. Chiapponi, and A. Luppi. 2023. *Salmonella* infection in pigs: Disease, prevalence, and a link between swine and human health. *Pathogens* 12:1267. doi:10.3390/pathogens12101267

Song, D., J. Lee, K. Kim, H. Oh, J. An, S. Chang, H. Cho, S. Park, K. Jeon, Y. Yoon, Y. Yoo, Y. Cho, and J. Cho. 2023. Effects of dietary supplementation of *Pediococcus pentosaceus* strains from kimchi in weaned piglet challenged with *Escherichia coli* and *Salmonella enterica*. *J. Anim. Sci. Technol.* 65:611-626. doi:10.5187/jast.2023.e31

Sreekumar, P. G., D. A. Ferrington, and R. Kannan. 2021. Glutathione metabolism and the novel role of mitochondrial GSH in retinal degeneration. *Antioxidants* 10:661. doi:10.3390/antiox10050661

Stephens, M., and P.-Y. von der Weid. 2020. Lipopolysaccharides modulate intestinal epithelial permeability and inflammation in a species-specific manner. *Gut Microbes* 11:421-432. doi:10.1080/19490976.2019.1629235

Sterndale, S. O., D. J. Evans, J. P. Mansfield, J. Clarke, S. Sahibzada, S. Abraham, M. O'Dea, D. W. Miller, J. C. Kim, and J. R. Pluske. 2019a. Correction to: effect of mucin 4 allele on susceptibility to experimental infection with enterotoxigenic F4 *Escherichia coli* in pigs fed experimental diets. *J. Anim. Sci. Biotechnol.* 10:71. doi:10.1186/s40104-019-0383-0

Sterndale, S. O., D. W. Miller, J. P. Mansfield, J. C. Kim, M. O'Dea, and J. R. Pluske. 2019b. Novel delivery methods for an enterotoxigenic Escherichia coli infection model in MUC4-locus sequenced weaner pigs. *J. Anim. Sci.* 97:4503-4508. doi:10.1093/jas/skz303

Stojanović, O., J. Altirriba, D. Rigo, M. Spiljar, E. Evrard, B. Roska, S. Fabbiano, N. Zamboni, P. Maechler, F. Rohner-Jeanrenaud, and M. Trajkovski. 2021. Dietary excess regulates absorption and surface of gut epithelium through intestinal PPARα. *Nat. Commun.* 12:7031. doi:10.1038/s41467-021-27133-7

Stoner, M. C., A. M. Scherr, J. A. Lee, L. G. Wolfe, and J. M. Kellum. 2000. Nitric oxide is a neurotransmitter in the chloride secretory response to serotonin in rat colon. *Surgery* 128:240-245. doi:10.1067/msy.2000.107608

Stothers, C. L., K. R. Burelbach, A. M. Owen, N. K. Patil, M. A. McBride, J. K. Bohannon, L. Luan, A. Hernandez, T. K. Patil, D. L. Williams, and E. R. Sherwood. 2021. β-glucan induces distinct and protective innate immune memory in differentiated macrophages. *J. Immunol.* 207:2785-2798. doi:10.4049/jimmunol.2100107

Su, W., T. Gong, Z. Jiang, Z. Lu, and Y. Wang. 2022a. The role of probiotics in alleviating postweaning diarrhea in piglets from the perspective of intestinal barriers. *Front. Cell. Infect. Microbiol.* 12:883107. doi:0.3389/fcimb.2022.883107

Su, W., Z. Jiang, C. Wang, Y. Zhang, T. Gong, F. Wang, M. Jin, Y. Wang, and Z. Lu. 2022b. Co-fermented defatted rice bran alters gut microbiota and improves growth performance, antioxidant capacity, immune status and intestinal permeability of finishing pigs. *Anim. Nutr.* 11:413-424. doi:10.1016/j.aninu.2022.07.008

Sun, Q., S. Zhang, X. Liu, Y. Huo, B. Su, and X. Li. 2020a. Effects of a probiotic intervention on *Escherichia coli* and high-fat diet-induced intestinal microbiota imbalance. *Appl. Microbiol. Biotechnol.* 104:1243-1257. doi:10.1007/s00253-019-10304-4

Sun, S., L. Luo, W. Liang, Q. Yin, J. Guo, A. M. Rush, Z. Lv, Q. Liang, M. A. Fischbach, J. L. Sonnenburg, D. Dodd, M. M. Davis, and F. Wang. 2020b. *Bifidobacterium* alters the gut microbiota and modulates the functional metabolism of T regulatory cells in the context of immune checkpoint blockade. *Proc. Natl. Acad. Sci. U.S.A.* 117:27509-27515. doi:10.1073/pnas.1921223117

Sun, W., W. Chen, K. Meng, L. Cai, G. Li, X. Li, and X. Jiang. 2023. Dietary supplementation with probiotic *Bacillus licheniformis* S6 improves intestinal integrity via modulating intestinal barrier function and microbial diversity in weaned piglets. *Biology* 12:238. doi:10.3390/biology12020238

Sun, Y., and S. W. Kim. 2017. Intestinal challenge with enterotoxigenic *Escherichia coli* in pigs, and nutritional intervention to prevent postweaning diarrhea. *Anim. Nutr.* 3:322-330. doi:10.1016/j.aninu.2017.10.001

Sun, Y., X. Wang, L. Li, C. Zhong, Y. Zhang, X. Yang, M. Li, and C. Yang. 2024. The role of gut microbiota in intestinal disease: from an oxidative stress perspective. *Front. Microbiol.* 15:1328324. doi:10.3389/fmicb.2024.1328324

Sundaram, V. K., C. Massaad, and J. Grenier. 2019. Liver X receptors and their implications in the physiology and pathology of the peripheral nervous system. *Int. J. Mol. Sci.* 20:4192. doi:10.3390/ijms20174192

Surendran Nair, M., M. A. Amalaradjou, and K. Venkitanarayanan. 2017. Antivirulence properties of probiotics in combating microbial pathogenesis. *Adv. Appl. Microbiol.* 98:1-29. doi:10.1016/bs.aambs.2016.12.001

Suzuki, T., J. Takahashi, and M. Yamamoto. 2023. Molecular basis of the KEAP1-NRF2 signaling pathway. *Mol. Cells* 46:133-141. doi:10.14348/molcells.2023.0028

Ta, L. P., E. Bujna, O. Antal, M. Ladányi, R. Juhász, A. Szécsi, S. Kun, S. Sudheer, V. K. Gupta, and Q. D. Nguyen. 2021. Effects of various polysaccharides (alginate, carrageenan, gums, chitosan) and their combination with prebiotic saccharides (resistant starch, lactosucrose, lactulose) on the encapsulation of probiotic bacteria *Lactobacillus casei* 01 strain. *Int. J. Biol. Macromol.* 183:1136-1144. doi:10.1016/j.ijbiomac.2021.04.170

Tan, J. K., L. Macia, and C. R. Mackay. 2023. Dietary fiber and SCFAs in the regulation of mucosal immunity. *J. Allergy Clin. Immunol.* 151:361-370. doi:10.1016/j.jaci.2022.11.007

Tang, C., B. Xie, Q. Zong, and Z. Sun. 2019. Proanthocyanidins and probiotics combination supplementation ameliorated intestinal injury in Enterotoxigenic Escherichia coli infected diarrhea mice. *J. Funct. Foods* 62:103521. doi:10.1016/j.jff.2019.103521

Tang, W., J. Liu, Y. Ma, Y. Wei, J. Liu, and H. Wang. 2021. Impairment of intestinal barrier function induced by early weaning via autophagy and apoptosis associated with gut microbiome and metabolites. *Front. Immunol.* 12:804870. doi:10.3389/fimmu.2021.804870

Tang, X., and K. Xiong. 2022. Intrauterine growth retardation affects intestinal health of suckling piglets via altering intestinal antioxidant capacity, glucose uptake, tight junction, and immune responses. *Oxid. Med. Cell. Longevity* 2022:2644205. doi:10.1155/2022/2644205

Tang, X., K. Xiong, R. Fang, and M. Li. 2022. Weaning stress and intestinal health of piglets: A review. *Front. Immunol.* 13:1042778. doi:10.3389/fimmu.2022.1042778

Tang, X., K. Xiong, Y. Zeng, and R. Fang. 2024. The mechanism of zinc oxide in alleviating diarrhea in piglets after weaning: A review from the perspective of intestinal barrier function. *Int. J. Mol. Sci.* 25:10040. doi:10.3390/ijms251810040

Tao, S., Y. Bai, T. Li, N. Li, and J. Wang. 2019. Original low birth weight deteriorates the hindgut epithelial barrier function in pigs at the growing stage. *FASEB J.* 33:9897-9912. doi:10.1096/fj.201900204RR

Temple, N. J. 2000. Antioxidants and disease: More questions than answers. *Nutr. Res.* 20:449-459. doi:10.1016/S0271-5317(00)00138-X

Ternhag, A., A. Törner, A. Svensson, K. Ekdahl, and J. Giesecke. 2008. Short- and long-term effects of bacterial gastrointestinal infections. *Emerg. Infect. Dis.* 14:143-148. doi:10.3201/eid1401.070524

Tian, T., Z. Wang, and J. Zhang. 2017. Pathomechanisms of oxidative stress in inflammatory bowel disease and potential antioxidant therapies. *Oxid. Med. Cell. Longevity* 2017:4535194. doi:10.1155/2017/4535194

Todorov, S. D., I. V. Ivanova, I. Popov, R. Weeks, and M. L. Chikindas. 2022. *Bacillus* spore-forming probiotics: benefits with concerns? *Crit. Rev. Microbiol.* 48:513-530. doi:10.1080/1040841X.2021.1983517

Tojo, R., A. Suárez, M. G. Clemente, C. G. de los Reyes-Gavilán, A. Margolles, M. Gueimonde, and P. Ruas-Madiedo. 2014. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. *World J. Gastroenterol.* 20:15163-15176. doi:10.3748/wjg.v20.i41.15163

Tonelli, C., I. I. C. Chio, and D. A. Tuveson. 2017. Transcriptional regulation by Nrf2. *Antioxid. Redox Signaling* 29:1727-1745. doi:10.1089/ars.2017.7342

Traber, M. G. 2024. Human Vitamin E deficiency, and what is and is not Vitamin E? *Free Radic. Biol. Med.* 213:285-292. doi:10.1016/j.freeradbiomed.2024.01.024

Uddin, M. K., M. R. Mahmud, S. Hasan, O. Peltoniemi, and C. Oliviero. 2023. Dietary micro-fibrillated cellulose improves growth, reduces diarrhea, modulates gut microbiota, and increases butyrate production in post-weaning piglets. *Sci. Rep.* 13:6194. doi:10.1038/s41598-023-33291-z

Upadhaya, S.-D., and I.-H. Kim. 2021. The impact of weaning stress on gut health and the mechanistic aspects of several feed additives contributing to improved gut health function in weanling piglets-A review. *Animals* 11:2418. doi:10.3390/ani11082418

Vachon, P. H., and J.-F. Beaulieu. 1992. Transient mosaic patterns of morphological and functional differentiation in the Caco-2 cell line. *Gastroenterology* 103:414-423. doi:10.1016/0016-5085(92)90829-N

Vairetti, M., L. G. Di Pasqua, M. Cagna, P. Richelmi, A. Ferrigno, and C. Berardo. 2021. Changes in glutathione content in liver diseases: an update. *Antioxidants* 10:364. doi:10.3390/antiox10030364

Valenzuela, A. 1991. The biological significance of malondialdehyde determination in the assessment of tissue oxidative stress. *Life Sci.* 48:301-309. doi:10.1016/0024-3205(91)90550-U

van Hoffen, E., A. Mercenier, K. Vidal, J. Benyacoub, J. Schloesser, A. Kardinaal, E. Lucas-van de Bos, I. van Alen, I. Roggero, K. Duintjer, A. Berendts, R. Albers, M. Kleerebezem, and S. ten Bruggencate. 2021. Characterization of the pathophysiological determinants of diarrheagenic *Escherichia coli* infection using

a challenge model in healthy adults. *Sci. Rep.* 11:6060. doi:10.1038/s41598-021-85161-1

Vannucci, F. A., and C. J. Gebhart. 2014. Recent advances in understanding the pathogenesis of *Lawsonia intracellularis* infections. *Vet. Pathol.* 51:465-477. doi:10.1177/0300985813520249

Vázquez-Meza, H., M. M. Vilchis-Landeros, M. Vázquez-Carrada, D. Uribe-Ramírez, and D. Matuz-Mares. 2023. Cellular compartmentalization, glutathione transport and its relevance in some pathologies. *Antioxidants* 12:834. doi:10.3390/antiox12040834

Velatooru, L. R., R. J. Abe, M. Imanishi, Y. J. Gi, K. A. Ko, K.-S. Heo, K. Fujiwara, N.-T. Le, and S. Kotla. 2021. Disturbed flow-induced FAK K152 SUMOylation initiates the formation of pro-inflammation positive feedback loop by inducing reactive oxygen species production in endothelial cells. *Free Radic. Biol. Med.* 177:404-418. doi:10.1016/j.freeradbiomed.2021.09.023

von Mentzer, A., and A.-M. Svennerholm. 2024. Colonization factors of human and animal-specific enterotoxigenic *Escherichia coli* (ETEC). *Trends Microbiol.* 32:448-464. doi:10.1016/j.tim.2023.11.001

Vona, R., L. Pallotta, M. Cappelletti, C. Severi, and P. Matarrese. 2021. The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. *Antioxidants* 10:201. doi:10.3390/antiox10020201

Vukmirović, Đ. M., S. M. Rakita, N. J. Spasevski, B. M. Kokić, V. V. Banjac, and I. S. Čabarkapa. 2017. A review of possibilities for control of *Salmonella* and other

pathogenic bacteria in pig feed. *Food Feed Res.* 2017:151-162. doi:10.5937/FFR1702151V

Wan, J., Y. Wu, Q. Pham, R. W. Li, L. Yu, M.-H. Chen, S. M. Boue, W. Yokoyama, B. Li, and T. T. Y. Wang. 2021. Effects of differences in resistant starch content of rice on intestinal microbial composition. *J. Agric. Food. Chem.* 69:8017-8027. doi:10.1021/acs.jafc.0c07887

Wang, H.-B., P.-Y. Wang, X. Wang, Y.-L. Wan, and Y.-C. Liu. 2012. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig. Dis. Sci.* 57:3126-3135. doi:10.1007/s10620-012-2259-4

Wang, H.-L., Y. He, S.-W.-M. Wang, M. Aziz-ur-Rahman, S.-Y. Zhang, C.-X. Shi, H.-M. Wang, and H.-W. Su. 2024a. Unlocking the potential of methionine: a dietary supplement for preventing colitis. *Food Funct.* 15:10373-10389. doi:10.1039/D4FO02883J

Wang, H., X. Huang, H. Tan, X. Chen, C. Chen, and S. Nie. 2022a. Interaction between dietary fiber and bifidobacteria in promoting intestinal health. *Food Chem.* 393:133407. doi:10.1016/j.foodchem.2022.133407

Wang, J., Y. Cao, C. Wang, and B. Sun. 2011. Wheat bran xylooligosaccharides improve blood lipid metabolism and antioxidant status in rats fed a high-fat diet. *Carbohydr. Polym.* 86:1192-1197. doi:10.1016/j.carbpol.2011.06.014

Wang, L., N. Liu, Y. Gao, J. Liu, X. Huang, Q. Zhang, Y. Li, J. Zhao, J. Wang, and G. Zhao. 2021. Surveillance and reduction control of *Escherichia coli* and

diarrheagenic *E. coli* during the pig slaughtering process in China. *Front. Vet. Sci.* 8:735076. doi:10.3389/fvets.2021.735076

Wang, M., L. Wang, X. Tan, L. Wang, X. Xiong, Y. Wang, Q. Wang, H. Yang, and Y. Yin. 2022b. The developmental changes in intestinal epithelial cell proliferation, differentiation, and shedding in weaning piglets. *Anim. Nutr.* 9:214-222. doi:10.1016/j.aninu.2021.11.006

Wang, M., S. Wichienchot, X. He, X. Fu, Q. Huang, and B. Zhang. 2019a. In vitro colonic fermentation of dietary fibers: Fermentation rate, short-chain fatty acid production and changes in microbiota. *Trends Food Sci. Technol.* 88:1-9. doi:10.1016/j.tifs.2019.03.005

Wang, S., Y. Fang, Y. Xu, B. Zhu, J. Piao, L. Zhu, L. Yao, K. Liu, S. Wang, Q. Zhang, L. Qin, and J. Wu. 2022c. The effects of different extraction methods on physicochemical, functional and physiological properties of soluble and insoluble dietary fiber from Rubus *chingii* Hu. fruits. *J. Funct. Foods* 93:105081. doi:10.1016/j.jff.2022.105081

Wang, W., D. Chen, B. Yu, Z. Huang, X. Mao, P. Zheng, Y. Luo, J. Yu, J. Luo, H. Yan, and J. He. 2020a. Effects of dietary inulin supplementation on growth performance, intestinal barrier integrity and microbial populations in weaned pigs. *Br. J. Nutr.* 124:296-305. doi:10.1017/S0007114520001130

Wang, X.-Y., T.-Q. Zhao, D.-P. Xu, X. Zhang, C.-J. Ji, and D.-L. Zhang. 2019b. The influence of porcine epidemic diarrhea virus on pig small intestine mucosal epithelial cell function. *Arch. Virol* 164:83-90. doi:10.1007/s00705-018-4061-x

Wang, Y., Y. Chen, X. Zhang, Y. Lu, and H. Chen. 2020b. New insights in intestinal oxidative stress damage and the health intervention effects of nutrients: A review. *J. Funct. Foods* 75:104248. doi:10.1016/j.jff.2020.104248

Wang, Y., Z. Zhang, M. Du, X. Ji, X. Liu, C. Zhao, X. Pang, E. Jin, A. Wen, S. Li, and F. Zhang. 2024b. Berberine alleviates ETEC-induced intestinal inflammation and oxidative stress damage by optimizing intestinal microbial composition in a weaned piglet model. *Front. Immunol.* 15:1460127. doi:10.3389/fimmu.2024.1460127

Wang, Z., Z. Li, Y. Ye, L. Xie, and W. Li. 2016. Oxidative stress and liver cancer: etiology and therapeutic targets. *Oxid. Med. Cell. Longevity* 2016:7891574. doi:10.1155/2016/7891574

Wapnir, R. A., and S. Teichberg. 2002. Regulation mechanisms of intestinal secretion: implications in nutrient absorption. *J. Nutr. Biochem.* 13:190-199. doi:10.1016/S0955-2863(02)00181-X

Wei, X., T. Tsai, S. Howe, and J. Zhao. 2021. Weaning induced gut dysfunction and nutritional interventions in nursery pigs: a partial review. *Animals* 11:1279. doi:10.3390/ani11051279

Weng, X., W. Wang, Q. Zhu, C. Liao, and G. Jiang. 2025. Concentrations, spatial distribution, and human exposure of synthetic phenolic antioxidants in indoor dust from ten provinces in China. *J. Environ. Sci.* 152:584-593. doi:10.1016/j.jes.2024.06.002

Wenk, C. 2001. The role of dietary fibre in the digestive physiology of the pig. *Anim. Feed Sci. Technol.* 90:21-33. doi:10.1016/S0377-8401(01)00194-8

WHO. 2021. WHO preferred product characteristics for vaccines against enterotoxigenic *Escherichia coli* (ETEC). Geneva: World Health Organization. License: CC BY-NC-SA 3.0 IGO.

Wijerathne, C. U. B., K. K. W. Au-Yeung, Y. L. Siow, and K. O. 2022. 5-Methyltetrahydrofolate attenuates oxidative stress and improves kidney function in acute kidney injury through activation of Nrf2 and antioxidant defense. *Antioxidants* 11:1046. doi:0.3390/antiox11061046

Williams, B. A., M. W. Verstegen, and S. Tamminga. 2001. Fermentation in the large intestine of single-stomached animals and its relationship to animal health. *Nutr. Res. Rev.* 14:207-228. doi:10.1079/NRR200127

Winiarska-Mieczan, A., M. Kwiecień, K. Jachimowicz-Rogowska, J. Donaldson, E. Tomaszewska, and E. Baranowska-Wójcik. 2023. Anti-inflammatory, antioxidant, and neuroprotective effects of polyphenols-polyphenols as an element of diet therapy in depressive disorders. *Int. J. Mol. Sci.* 24:2258. doi:10.3390/ijms24032258

Worliczek, H. L., W. Gerner, A. Joachim, H.-C. Mundt, and A. Saalmüller. 2009. Porcine Coccidiosis - Investigations on the Cellular Immune Response against *Isospora suis*. *Parasitol. Res.* 105:151-156. doi:10.1007/s00436-009-1506-x

Woźniak, D., W. Cichy, J. Przysławski, and S. Drzymała-Czyż. 2021. The role of microbiota and enteroendocrine cells in maintaining homeostasis in the human digestive tract. *Adv. Med. Sci.* 66:284-292. doi:10.1016/j.advms.2021.05.003

Wu, H., X. Wang, X. Zhou, S. Chen, W. Mai, H. Huang, Z. You, S. Zhang, X. Zhang, and B. Lu. 2021a. Osteomyelitis and septic arthritis due to *Burkholderia pseudomallei*:

a 10-year retrospective melioidosis study from South China. *Front. Cell. Infect. Microbiol.* 11:654745. doi:10.3389/fcimb.2021.654745

Wu, T., Y. Shi, Y. Zhang, M. Zhang, L. Zhang, Z. Ma, D. Zhao, L. Wang, H. Yu, Y. Hou, and J. Gong. 2021b. *Lactobacillus rhamnosus* LB1 alleviates enterotoxigenic *Escherichia coli*-induced adverse effects in piglets by improving host immune response and anti-oxidation stress and restoring intestinal integrity. *Front. Cell. Infect. Microbiol.* 11:724401. doi:10.3389/fcimb.2021.724401

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

Wu, Y., S. Hu, C. Wu, F. Gu, and Y. Yang. 2022. Probiotics: potential novel therapeutics against fungal infections. *Front. Cell. Infect. Microbiol.* 11:793419. doi:10.3389/fcimb.2021.793419

Xia, B., J. Yu, T. He, X. Liu, J. Su, M. Wang, J. Wang, and Y. Zhu. 2020. *Lactobacillus johnsonii* L531 ameliorates enteritis via elimination of damaged mitochondria and suppression of SQSTM1-dependent mitophagy in a *Salmonella* infantis model of piglet diarrhea. *FASEB J.* 34:2821-2839. doi:10.1096/fj.201901445RRR

Xie, F., W. Yang, M. Xing, H. Zhang, and L. Ai. 2023. Natural polyphenols-gut microbiota interactions and effects on glycolipid metabolism via polyphenols-gut-brain axis: A state-of-the-art review. *Trends Food Sci. Technol.* 140:104171. doi:10.1016/j.tifs.2023.104171

Xie, W., L. Song, X. Wang, Y. Xu, Z. Liu, D. Zhao, S. Wang, X. Fan, Z. Wang, and C. Gao. 2021. A bovine lactoferricin-lactoferrampin-encoding *Lactobacillus reuteri* CO21 regulates the intestinal mucosal immunity and enhances the protection of piglets against enterotoxigenic *Escherichia coli* K88 challenge. *Gut Microbes* 13:1956281. doi:10.1080/19490976.2021.1956281

Xu, E., C. Chen, J. Fu, L. Zhu, J. Shu, M. Jin, Y. Wang, and X. Zong. 2021a. Dietary fatty acids in gut health: Absorption, metabolism and function. *Anim. Nutr.* 7:1337-1344. doi:10.1016/j.aninu.2021.09.010

Xu, H., J. Gong, P. Lu, P. Azevedo, L. Li, H. Yu, and C. Yang. 2024. Functional evaluation of *Bacillus licheniformis* PF9 for its potential in controlling enterotoxigenic *Escherichia coli* in weaned piglets. *Transl. Anim. Sci.* 8:txae050. doi:10.1093/tas/txae050

Xu, S., Y. He, L. Lin, P. Chen, M. Chen, and S. Zhang. 2021b. The emerging role of ferroptosis in intestinal disease. *Cell Death Dis.* 12:289. doi:10.1038/s41419-021-03559-1

Yamamoto, T., H. Kuramoto, and M. Kadowaki. 2007. Downregulation in aquaporin 4 and aquaporin 8 expression of the colon associated with the induction of allergic diarrhea in a mouse model of food allergy. *Life Sci.* 81:115-120. doi:10.1016/j.lfs.2007.04.036

Yan, E., J. Zhang, H. Han, J. Wu, Z. Gan, C. Wei, L. Zhang, C. Wang, and T. Wang. 2020. Curcumin alleviates IUGR jejunum damage by increasing antioxidant capacity through Nrf2/Keap1 pathway in growing pigs. *Animals* 10:41. doi:10.3390/ani10010041

Yan, N., and A. Meister. 1990. Amino acid sequence of rat kidney gamma-glutamylcysteine synthetase. *J. Biol. Chem*. 265:1588-1593. doi:10.1016/S0021-9258(19)40057-4

Yan, W., J. Luo, Z. Yu, and B. Xu. 2024. A critical review on intestinal mucosal barrier protection effects of dietary polysaccharides. *Food Funct.* 15:481-492. doi:10.1039/D3FO03412G

Yang, G.-Y., Y.-H. Zhu, W. Zhang, D. Zhou, C.-C. Zhai, and J.-F. Wang. 2016. Influence of orally fed a select mixture of *Bacillus* probiotics on intestinal T-cell migration in weaned *MUC4* resistant pigs following *Escherichia coli* challenge. *Vet. Res.* 47:71. doi:10.1186/s13567-016-0355-8

Yang, K. M., Z. Y. Jiang, C. T. Zheng, L. Wang, and X. F. Yang. 2014. Effect of *Lactobacillus plantarum* on diarrhea and intestinal barrier function of young piglets challenged with enterotoxigenic *Escherichia coli* K88. *J. Anim. Sci.* 92:1496-1503. doi:10.2527/jas.2013-6619

Yang, S., and G. Lian. 2020. ROS and diseases: role in metabolism and energy supply. *Mol. Cell. Biochem*. 467:1-12. doi:10.1007/s11010-019-03667-9

Yang, X., A. Li, X. Li, L. Sun, and Y. Guo. 2020. An overview of classifications, properties of food polysaccharides and their links to applications in improving food textures. *Trends Food Sci. Technol.* 102:1-15. doi:10.1016/j.tifs.2020.05.020

Yang, Y., A. V. Bazhin, J. Werner, and S. Karakhanova. 2013. Reactive oxygen species in the immune system. *Int. Rev. Immunol.* 32:249-270. doi:10.3109/08830185.2012.755176

Ye, C., Y. Chen, R. Yu, M. Zhao, R. Yin, Y. Qiu, S. Fu, Y. Liu, and Z. Wu. 2024. Baicalin-aluminum complex on the regulation of IPEC-1 infected with enterotoxigenic *Escherichia coli*. *Heliyon* 10:e33038. doi:10.1016/j.heliyon.2024.e33038

Ye, S., B. R. Shah, J. Li, H. Liang, F. Zhan, F. Geng, and B. Li. 2022. A critical review on interplay between dietary fibers and gut microbiota. *Trends Food Sci. Technol.* 124:237-249. doi:10.1016/j.tifs.2022.04.010

Ye, Z., Y.-J. Xu, and Y. Liu. 2021. Influences of dietary oils and fats, and the accompanied minor content of components on the gut microbiota and gut inflammation: A review. *Trends Food Sci. Technol.* 113:255-276. doi:10.1016/j.tifs.2021.05.001

Yi, Q., J. Liu, Y. Zhang, H. Qiao, F. Chen, S. Zhang, and W. Guan. 2021. Anethole attenuates enterotoxigenic *Escherichia coli*-induced intestinal barrier disruption and intestinal inflammation via modification of TLR signaling and intestinal microbiota. *Front. Microbiol.* 12:647242. doi:10.3389/fmicb.2021.647242

Yin, J., M. Liu, W. Ren, J. Duan, G. Yang, Y. Zhao, R. Fang, L. Chen, T. Li, and Y. Yin. 2015. Effects of dietary supplementation with glutamate and aspartate on diquat-induced oxidative stress in piglets. *PLoS One* 10:e0122893. doi:10.1371/journal.pone.0122893

Yin, J., M. Wu, H. Xiao, W. Ren, J. Duan, G. Yang, T. Li, and Y. Yin. 2014. Development of an antioxidant system after early weaning in piglets. *J. Anim. Sci.* 92:612-619. doi:10.2527/jas.2013-6986

Yu, C., Z. Wang, S. Tan, Q. Wang, C. Zhou, X. Kang, S. Zhao, S. Liu, H. Fu, Z. Yu, and A. Peng. 2016. Chronic kidney disease induced intestinal mucosal barrier damage

associated with intestinal oxidative stress injury. *Gastroenterol. res. pract.* 2016:6720575. doi:10.1155/2016/6720575

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

Yu, S., Y. Sun, X. Shao, Y. Zhou, Y. Yu, X. Kuai, and C. Zhou. 2022a. Leaky gut in IBD: Intestinal barrier–gut microbiota interaction. *J. Microbiol. Biotechnol.* 32:825-834. doi:10.4014/jmb.2203.03022

Yu, X., Z. Cui, S. Qin, R. Zhang, Y. Wu, J. Liu, and C. Yang. 2022b. Effects of *Bacillus licheniformis* on growth performance, diarrhea incidence, antioxidant capacity, immune function, and fecal microflora in weaned piglets. *Animals* 12:1609. doi:10.3390/ani12131609

Yu, X., L. Zhu, T. Wang, and J. Chen. 2023. Immune microenvironment of cholangiocarcinoma: Biological concepts and treatment strategies. *Front. Immunol.* 14:1037945. doi:10.3389/fimmu.2023.1037945

Yuan, S., D. Chen, K. Zhang, and B. Yu. 2007. Effects of oxidative stress on growth performance, nutrient digestibilities and activities of antioxidative enzymes of weanling pigs. *Asian-Australas. J. Anim. Sci.* 20:1600-1605. doi:10.5713/ajas.2007.1600

Yue, Y., Z. He, Y. Zhou, R. P. Ross, C. Stanton, J. Zhao, H. Zhang, B. Yang, and W. Chen. 2020. *Lactobacillus plantarum* relieves diarrhea caused by enterotoxin-producing

Escherichia coli through inflammation modulation and gut microbiota regulation. *Food Funct.* 11:10362-10374. doi:10.1039/D0FO02670K

Yun, B., M. King, M. S. Draz, T. Kline, and A. Rodriguez-Palacios. 2022. Oxidative reactivity across kingdoms in the gut: Host immunity, stressed microbiota and oxidized foods. *Free Radic. Biol. Med.* 178:97-110. doi:10.1016/j.freeradbiomed.2021.11.009

Yun, W., M. Song, J. Lee, H. Oh, J. An, G. Kim, S. Lee, S. Lee, H. B. Kim, and J. Cho. 2020. Arginine addition in a diet for weaning pigs can improve the growth performance under heat stress. *J. Anim. Sci. Technol.* 62:460-467. doi:10.5187/jast.2020.62.4.460

Zeng, N., F. Wu, J. Lu, X. Li, S. Lin, L. Zhou, Z. Wang, G. Wu, Q. Huang, D. Zheng, J. Gao, S. Wu, X. Chen, M. Chen, F. Meng, H. Shang, Y. He, P. Chen, H. Wei, Z. Li, and H. Zhou. 2024. High-fat diet impairs gut barrier through intestinal microbiota-derived reactive oxygen species. *Sci. China Life Sci.* 67:879-891. doi:10.1007/s11427-022-2283-4

Zeng, Y., H. Wang, R. Ruan, Y. Li, Z. Liu, C. Wang, and A. Liu. 2022. Effect of stocking density on behavior and pen cleanliness of grouped growing pigs. *Agriculture* 12:418. doi:10.3390/agriculture12030418

Zganiacz, A., M. Santosuosso, J. Wang, T. Yang, L. Chen, M. Anzulovic, S. Alexander, B. Gicquel, Y. Wan, J. Bramson, M. Inman, and Z. Xing. 2004. TNF-α is a critical negative regulator of type 1 immune activation during intracellular bacterial infection. *J. Clin. Investig.* 113:401-413. doi:10.1172/JCI18991

Zhang, M., C. Huang, J. Ou, F. Liu, S. Ou, and J. Zheng. 2024. Glyoxal in foods: Formation, metabolism, health hazards, and its control strategies. *J. Agric. Food. Chem.* 72:2434-2450. doi:10.1021/acs.jafc.3c08225

Zhang, N., M. Jin, K. Wang, Z. Zhang, N. P. Shah, and H. Wei. 2022a. Functional oligosaccharide fermentation in the gut: Improving intestinal health and its determinant factors-A review. *Carbohydr. Polym.* 284:119043. doi:10.1016/j.carbpol.2021.119043

Zhang, W., C. Bao, J. Wang, J. Zang, and Y. Cao. 2020. Administration of *Saccharomyces boulardii* mafic-1701 improves feed conversion ratio, promotes antioxidant capacity, alleviates intestinal inflammation and modulates gut microbiota in weaned piglets. *J. Anim. Sci. Biotechnol.* 11:112. doi:10.1186/s40104-020-00516-4

Zhang, X., L. Hou, Z. Guo, G. Wang, J. Xu, Z. Zheng, K. Sun, and F. Guo. 2023. Lipid peroxidation in osteoarthritis: focusing on 4-hydroxynonenal, malondialdehyde, and ferroptosis. *Cell Death Discov.* 9:320. doi:10.1038/s41420-023-01613-9

Zhang, Y., P. Tan, Y. Zhao, and X. Ma. 2022b. Enterotoxigenic *Escherichia coli*: Intestinal pathogenesis mechanisms and colonization resistance by gut microbiota. *Gut Microbes* 14:2055943. doi:10.1080/19490976.2022.2055943

Zhang, Z., H. Zhang, T. Chen, L. Shi, D. Wang, and D. Tang. 2022c. Regulatory role of short-chain fatty acids in inflammatory bowel disease. *Cell. Commun. Signal.* 20:64. doi:10.1186/s12964-022-00869-5

Zhao, J., Y. Bai, G. Zhang, L. Liu, and C. Lai. 2020. Relationship between dietary fiber fermentation and volatile fatty acids' concentration in growing pigs. *Animals* 10:263. doi:10.3390/ani10020263

Zhao, J., P. Liu, Y. Wu, P. Guo, L. Liu, N. Ma, C. Levesque, Y. Chen, J. Zhao, and J. Zhang. 2018. Dietary fiber increases butyrate-producing bacteria and improves the growth performance of weaned piglets. *J. Agric. Food Chem.* 66:7995-8004. doi:10.1021/acs.jafc.8b02545

Zhao, J., S. Wan, N. Sun, P. Sun, Y. Sun, A. Khan, J. Guo, X. Zheng, K. Fan, W. Yin, and H. Li. 2021. Damage to intestinal barrier integrity in piglets caused by porcine reproductive and respiratory syndrome virus infection. *Vet. Res.* 52:93. doi:10.1186/s13567-021-00965-3

Zhao, Z., S. Xu, W. Zhang, D. Wu, and G. Yang. 2022. Probiotic *Escherichia coli* NISSLE 1917 for inflammatory bowel disease applications. *Food Funct.* 13:5914-5924. doi:10.1039/D2FO00226D

Zhen, Y., and H. Zhang. 2019. NLRP3 inflammasome and inflammatory bowel disease. *Front. Immunol.* 10:276. doi:10.3389/fimmu.2019.00276

Zheng, J., A. A. Ahmad, Y. Yang, Z. Liang, W. Shen, M. Feng, J. Shen, X. Lan, and X. Ding. 2022. *Lactobacillus rhamnosus* CY12 enhances intestinal barrier function by regulating tight junction protein expression, oxidative stress, and inflammation response in lipopolysaccharide-induced Caco-2 cells. *Int. J. Mol. Sci.* 23:11162. doi:10.3390/ijms231911162

Zheng, M., Y. Liu, G. Zhang, Z. Yang, W. Xu, and Q. Chen. 2023. The applications and mechanisms of superoxide dismutase in medicine, food, and cosmetics. *Antioxidants* 12:1675. doi:10.3390/antiox12091675

Zheng, S., Y. Zhang, Q. Chen, X. Fu, Q. Huang, Z. Bin, H. Dong, and C. Li. 2024. Exploring the synergistic benefits of insoluble dietary fiber and bound phenolics: Unveiling the role of bound phenolics in enhancing bioactivities of insoluble dietary fiber. *Trends Food Sci. Technol.* 149:104554. doi:10.1016/j.tifs.2024.104554

Zhou, J.-y., D.-g. Huang, C.-q. Gao, H.-c. Yan, S.-g. Zou, and X.-q. Wang. 2021a. Heat-stable enterotoxin inhibits intestinal stem cell expansion to disrupt the intestinal integrity by downregulating the Wnt/β-catenin pathway. *Stem Cells* 39:482-496. doi:10.1002/stem.3324

Zhou, J., M. Li, Q. Chen, X. Li, L. Chen, Z. Dong, W. Zhu, Y. Yang, Z. Liu, and Q. Chen. 2022. Programmable probiotics modulate inflammation and gut microbiota for inflammatory bowel disease treatment after effective oral delivery. *Nat. Commun.* 13:3432. doi:10.1038/s41467-022-31171-0

Zhou, S.-X., L.-P. Wang, M.-Y. Liu, H.-Y. Zhang, Q.-B. Lu, L.-S. Shi, X. Ren, Y.-F. Wang, S.-H. Lin, C.-H. Zhang, M.-J. Geng, X.-A. Zhang, Y.-L. Zhu, Z.-J. Li, L.-Q. Fang, W. Liu, and W.-Z. Yang. 2021b. Characteristics of diarrheagenic Escherichia coli among patients with acute diarrhea in China, 2009–2018. *J. Infect.* 83:424-432. doi:10.1016/j.jinf.2021.08.001

Zou, J., B. Chassaing, V. Singh, M. Pellizzon, M. Ricci, M. D. Fythe, M. V. Kumar, and A. T. Gewirtz. 2018. Fiber-mediated nourishment of gut microbiota protects against

diet-induced obesity by restoring IL-22-mediated colonic health. *Cell Host Microbe* 23:41-53. doi:10.1016/j.chom.2017.11.003

Zwick, R. K., P. Kasparek, B. Palikuqi, S. Viragova, L. Weichselbaum, C. S. McGinnis, K. L. McKinley, A. Rathnayake, D. Vaka, V. Nguyen, C. Trentesaux, E. Reyes, A. R. Gupta, Z. J. Gartner, R. M. Locksley, J. M. Gardner, S. Itzkovitz, D. Boffelli, and O. D. Klein. 2024. Epithelial zonation along the mouse and human small intestine defines five discrete metabolic domains. *Nat. Cell Biol.* 26:250-262. doi:10.1038/s41556-023-01337-z