Gastrointestinal Tract Barrier Efficiency: Function and Threats

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Abstract

The gastrointestinal tract (GIT) serves as a massive interface with the outside environment and physical barriers in the upper part, such as acids in the stomach and antimicrobial factors in saliva and other excreta, it is inevitably colonized by a multitude of micro-organisms which must be prevented from entering the sterile body-proper along with the nutrients. The absorptive intestinal epithelial cells are therefore assisted along the GIT by other epithelial cells that provide mechanical, biochemical, and biological barriers and by various immune cells that distinguish a small number of potential foes from a large number of friends. Both the failure to engage the small number of pathogens and the failure to ignore the large amounts of harmless antigens could lead to detrimental inflammatory responses and loss of health. The GIT and its associated barrier and immune functions evolve with time and can be altered by certain nutrients or other dietary additives. The purpose of this chapter is to give an overview of fundamental properties of the GIT barrier distal of the stomach and its associated immune system, where possible with emphasis on chickens; how these evolve during their lifespan; and how these functions could be affected by functional nutrients.

Keywords

Gut barrier functions · Inflammatory response · Immune system · Functional nutrients

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M. H. Kogut, G. Zhang (eds.), *Gut Microbiota, Immunity, and Health in Production Animals*, The Microbiomes of Humans, Animals, Plants, and the Environment 4,
https://doi.org/10.1007/978-3-030-90303-9_2

2.1 Introduction

Modern poultry and swine production integrates ultra-fast-growing breeds with relatively low-quality raw dietary feedstuff, stressful rearing conditions, and significant pathogenic challenges within difficult sanitary conditions. The animals thus need to balance highly efficient nutrient uptake with efficient exclusion of pathogenic challenges while tolerating harmless antigens. The ever-shorter lifespan of production animals, especially broiler chickens, means that there is little time to develop a highly performing gastrointestinal tract (GIT) barrier and a highly efficient immune system.

The consequences of GIT barrier breakdown, whatever the reason may be, can be severe as they may expose the body-proper to large amounts of pro-inflammatory material. As a consequence, the animal (if it survives) needs to divert valuable resources to combat invading micro-organisms, clear all sorts of antigens, and repair damaged tissue. During such inflammatory responses, less resources are available for growth and appetite is generally suppressed. The gut barrier is also an important endocrine organ that regulates insulin sensitivity and food intake, among others, and affects behavior by signaling to the central nervous system with its own signaling molecules and through bacterial metabolites it acquires from the nearby microbiome.

The GIT barrier is both a mechanical and an immunological barrier and some dietary components or additives may strengthen the one and thereby unintentionally weakening the other. For example, dietary immune stimulants may strengthen the antimicrobial activity of intestinal immune cells but in absence of pathogens this would cost energy and perhaps even create a chronic low-grade inflammatory response which could weaken the barrier.

In this chapter, some features of the GIT barrier will be discussed as will some threats to these features and nutritional interventions aimed at alleviating the threats.

2.2 Gut Barrier Function

2.2.1 GIT Barrier Composition

If it were not for a healthy gut barrier, micro-organisms and dietary and microbial antigens (collectively "antigens") would enter the body via gut-draining lymph or gut-draining portal blood. The mesenteric lymph nodes (Mueller and Macpherson 2006) and the liver (Knolle and Gerken 2000) are the ultimate firewalls for these ports of entry and clear most antigens without inflammatory responses. Chicken lymph nodes are not as well defined as their mammalian counterparts, so the chicken's lymphatic firewall (if any) remains to be defined.

Before antigens can enter portal blood or mesenteric lymph, they must first enter the lamina propria of the GIT, a layer of connective tissue upon which the basement membrane and the mucosal epithelial layer are stacked. Here they must escape the many macrophages (Bain and Mowat 2014; Lee et al. 1985) patrolling the tissue. In contrast to most macrophages elsewhere in the body, GIT macrophages are

surprisingly tolerant toward the many antigens they may encounter (Smythies et al. 2005), which prevents uncontrolled inflammatory responses to the inevitably frequently infiltrating antigens. Failure to adopt this phenotype may cause chronic inflammatory bowel diseases (Na et al. 2019). How gut macrophages adopt this important phenotype once they enter the tissue from the bloodstream is not entirely clear but it can be induced by exogenous factors such as butyric acid (Schulthess et al. 2019) or endogenous factors such as Transforming Growth Factor beta 2 (TGFb2) (Maheshwari et al. 2011). Their phagocytic and bacteriocidic capability remains fully intact, however, and they express significant amounts of antimicrobial peptides (AMP) (Schulthess et al. 2019; Sunkara et al. 2011). Intestinal macrophages are also essential for maintenance and repair of the gut barrier, because they clear dead (apoptotic) cells and promote epithelial repair (Birkl et al. 2019; Cosín-Roger et al. 2016).

Dendritic cells, which are closely related to macrophages, also take up antigens in the lamina propria but then move to gut-draining lymph nodes or Gut Associated Lymphoid Tissue ("GALT") such as Peyer's Patches ("PP") where they present the acquired antigens to cognate T-lymphocytes. Signals that the cells had experienced at time of antigen uptake then dictate the type of response they instill into the lymphocytes: When dietary antigens were sampled, for example, signals were likely benign and cognate T-cells should differentiate into regulatory T-cells clones which suppress immune responses to these antigens ("oral tolerance") (Pabst and Mowat 2012). If alarm signals were present during sampling, for example because of tissue damage during an infection, then the T-cells will develop into effector cells hunting for the antigen or into helper cells ("Th cells") that will instruct other immune cells to combat the antigen when it shows up again. Lymphocyte clones "trained" by dendritic cells then either remain in the GALT where they could respond to antigens that cross epithelial "Microfold" cells ("M cells") (Jeurissen et al. 1999) in a rather controlled manner or move into the lamina propria where they could encounter antigens that breach the barrier in an uncontrolled manner. Altogether, these adaptive immune responses enable a more fine-tuned response to antigens than the more aspecific response of macrophages. One such response consists of production of immunoglobulin A (IgA) by lamina propria B-lymphocytes which is then shuttled through epithelial cells into the gut lumen (Mueller and Macpherson 2006; Brandtzaeg 2013). Secretory IgA thus adds another layer to the gut barrier.

Before entering the lamina propria, antigens must first have crossed the intestinal epithelium, a single layer of highly polarized cells that are tightly connected with each other with several extracellular and membrane-anchored proteins. These "Tight Junctions" (TJ) are impermeable for even the smallest molecules (Zihni et al. 2016) and separate a functionally and morphologically distinct apical (lumen-facing) surface from the basolateral surface. Loss of TJ integrity would lead to uncontrolled paracellular leakage (i.e., between epithelial cells) of antigens which could set in motion an inflammatory response. The epithelial TJ is the barrier layer that has perhaps received the most attention.

Before being able to move across or in between epithelial cells, the antigens must first have crossed the mucus on top of the epithelium. This layer consists of a mesh of

mucins, a group of glycoproteins excreted by epithelial Goblet cells. These mucins then get entangled with other mucin proteins which are attached to the apical surface of most epithelial cells. Mucus production requires significant amounts of threonine so it must be regulated to avoid wasting of this precious nutrient (Zhang et al. 2017b; Munasinghe et al. 2017). The mucus layer is hard to penetrate by bacteria unless they express flagella. Numerous antimicrobial factors within the mucus layer, such as Serum Amyloid, are produced by epithelial cells themselves and can further reinforce the mucus layer's barrier effect (Eckhardt et al. 2010), defensins (Ramasamy et al. 2012; Veldhuizen et al. 2008; Wehkamp et al. 2005), REGIIIg (Mukherjee et al. 2014), and cathelicidins (Achanta et al. 2012) while others (such as IgA) are produced elsewhere and are subsequently transcytosed across the epithelial cells into the gut lumen. Intestinal epithelial cells also possess membrane-bound alkaline phosphatase, which readily detoxifies LPS (Bates et al. 2007; Lallès 2014), and these cells thus form another protective layer.

Another feature of intestinal epithelial cells, which is becoming increasingly appreciated, is that they help orchestrate mucosal immune responses. This goes beyond merely releasing potent chemokines (such as Interleukin 8 (Il-8)) during infections to attract immune cells: Depending on the type of threat, epithelial cells release cytokines that may skew the subsequent immune response. Some dietary factors trigger epithelial release of TSLP, IL-25, or IL-33 (Li et al. 2013) which would be optimal for a Th2 response towards parasites. Conversely, segmented filamentous bacteria in the intestine cause epithelial cells to release SAA (serum amyloid A) which induces a Th17 bias (Okumura and Takeda 2017), a preferable response to extracellular bacterial pathogens.

The first barrier, however, that an antigen must breach before it even reaches the mucus is not the least and consists of the gut microbiome. It is composed of hundreds of different species of micro-organisms (mainly bacteria) which help directly or indirectly reduce the risk of infections with pathogenic species. One protective mechanism consists of pathogen exclusion (Callaway et al. 2008; Ceccarelli et al. 2017; Mead 2000), in that a healthy microbiome may occupy pathogens' niches, compete for their nutrients, outright kill them, or prevent access to receptors on the apical surface of epithelial cells. The gut microbiome can be modulated by the diet or by supplements such as pre- and probiotics and such manipulations are attractive strategies to improve GIT barrier function (Fouhse et al. 2016; Sanders et al. 2019; Kogut 2018).

2.2.2 GIT Barrier Assessment

When GIT barrier function is discussed, it is often done so in terms of leakage of material from the gut into the bloodstream, the extent of which can be predicted with a variety of methods. Some are active in the sense that they require oral administration of macromolecular markers and subsequent measurement of these markers in urine or blood. Two markers are usually administered in a certain ratio. Marker A is only absorbable in a paracellular manner across leaky tight junctions (or across an

epithelial wound) whereas marker B is readily absorbed transcellularly. A high ratio of A to B in urine would indicate strong intestinal leakage. In human studies a combination of the non-metabolizable sugars Lactulose (or Rhamnose) and Mannitol usually serves as paracellular and transcellular tracers, respectively. The method has revealed increased intestinal permeability in, for example, Crohn's (Andre et al. 1988) and celiac (Juby et al. 1989) disease and acute intestinal infections (Zhang et al. 2000). The method has also been used in swine to demonstrate increased gut leakiness during weaning (Hu et al. 2012b) or diet-induced dysbiosis (Li et al. 2018a), for example. Its application in chickens has also been described (Gilani et al. 2017) but requires blood sampling. Given this limitation, it may be easier to use markers that are easier to measure in blood such as fluorescein-isothiocyanate-labeled dextran ("FITC"-dextran). However, in absence of a second marker to control for gastric emptying one might miss peak serum levels of orally administered FITC dextran.

"Passive" assessment of intestinal permeability is based on measuring blood levels of markers of epithelial damage, such as enterocyte-restricted Diamine Oxidase (DAO) (Gilani et al. 2017; Hu et al. 2012a; Honzawa et al. 2011) or Fatty-Acid Binding Protein (López-Colom et al. 2019). Blood levels of Zonulin-1, a regulator of TJ function, have also been found to correlate with intestinal permeability (Fasano et al. 2000).

In vitro models are useful to study cellular and molecular aspects of intestinal barrier function and their regulation by antigens, toxins, or cytokines. The most widely used model consists of Caco-2 cells grown on permeable filter supports. Though of colonic origin, after about 2 weeks this human cancer cell line will differentiate into small-intestine-like epithelial cells with strong TJ the strength of which can be assessed by measuring trans-epithelial electrical resistance (TEER) or by measuring leakage of fluorescent FITC dextran or Lucifer yellow. The cells' TJ are sensitive to exogenous cytokines, toxins, or various antigens which enables pre-clinical studies to the efficacy of additives. They can also be co-cultured with macrophage-like cells and other immune cells (Satsu et al. 2006) to study the effect of additives in more complex models (Olejnik et al. 2016). IPEC-J2 cells are comparable porcine cell lines of jejunal origin (Yan and Ajuwon 2017). Chicken in vitro studies mostly rely on freshly isolated primary cells which cannot be maintained in culture very long (Byrne et al. 2007). The recently discovered ability to cultured intestinal organoids has revolutionized research into regulation of cell differentiation during gut development (Wallach and Bayrer 2017) and has been developed for swine (Wellock et al. 2009) and chickens (Cowieson et al. 2004). This technique could accelerate research and could reduce the use of experimental animals.

2.2.3 Gut Barrier Threats

As outlined above, a healthy GIT barrier is essential for good health and performance because it prevents uncontrolled inflammatory responses to the many

antigens that reside in the gut. The GIT barrier is constantly experiencing different threats. When any of these threats lead to GIT barrier rupture, the result may be inflammation in the intestine or in the rest of the body, leading to reduced growth and resilience. Restoration of gut barrier function, regardless of whether it is a cause or consequence of a disease, could improve health. In the following some of the more common stresses in production animals are discussed as are some mitigation strategies involving dietary supplements.

2.2.3.1 Emotional Stress

One important risk factor affecting GIT barrier function in early weaned piglets is emotional stress, of which the gut stress hormone signaling is partially mediating GIT barrier impairment (Moeser et al. 2007, 2017). Some additives or supplements have been shown to improve gut barrier function during weaning stress, such as butyric acid (Huang et al. 2015). This bacterial metabolite is an important energy source for (colonic) epithelial cells and has a variety of beneficial effects on intestinal cells (Bedford and Gong 2018). It is often added to the diet of production animals as a sodium salt or can be coated to mask odor and reduce its absorption in the upper GIT. Coated sodium butyrate improves ileal villous architecture in weaned piglets, a proxy for GIT barrier function (Upadhaya et al. 2020). Another strategy of reduction of upper GIT absorption of butyric acid is by covalently linking to glycerol, which increases exposure of distal parts of the GIT to butyric acid. Other feed additives believed to strengthen weaning piglets' GIT barrier function, such as certain amino acids or plant extracts, may or may not exert their beneficial effect directly on gut epithelial cells (Xiong et al. 2019).

The GIT contains a sizeable nervous system, which communicates with the central nervous system and controls motility and neuroendocrine functions in the gut. The relationship between the central and intestinal nervous systems is complex, as is the relation between the intestinal nervous system and the mucosal immune system (Powell et al. 2017). On top of this, all these functions are affected by metabolites produced by the gut microbiome (Cryan et al. 2019). These components of the so-called microbiota–gut–brain axis can affect each other (Cryan et al. 2019) and perturbations in one can affect the function of all others. One could argue that stimulation of production of beneficial metabolites in the GIT, for example by improving the intestinal microbiome, could help improve well-being of animals and help them cope with emotional stress. This is an active area of research, not in the least in the feed additive industry, but is still in its early stages.

An important emotional stressor for chickens is their high packing density and there are indications that this may affect gut barrier function (Goo et al. 2019). Heat stress is another important source of stress in the poultry industry (Goo et al. 2019; Song et al. 2014; Tabler et al. 2020). Its impact can be blunted to some extent with additives conceivably acting on the GIT barrier itself, such as probiotics (Song et al. 2014), butyric acid (Abdelqader and Al-Fataftah 2016), or additives with a potentially broader target (Shakeri et al. 2019; Zhang et al. 2017a; Wu et al. 2018). When the GIT suffers from chronic oxidative stress, due to an imbalance in production and clearance of reactive oxygen species (ROS), this results in various intestinal

diseases, including enteric infections, inflammatory diseases, and in human's inflammatory bowel disease (Wang et al. 2020). Numerous natural antioxidants in "functional foods" that include fat-soluble (vitamin E, carotenoids), water-soluble (ascorbic acid), proteins, selenium (Se), and phytochemicals have been documented to play a key role in maintaining chicken health through protecting the intestinal health and restoring damage caused by oxidative stress (Surai et al. 2019). Within the body an antioxidant defense system utilizes various antioxidants that work together with Se playing a huge role expressing antioxidants modulatory effects in breeders, newly hatched chicks, and postnatal chickens as well. At the molecular level, most stresses are associated with overproduction of free radicals and oxidative stress (Surai 2016). One main source of free radicals is electron transport chain of mitochondria which can lose up to 3% of oxygen from the energy production process and become free radicals damaging various biological systems including lipids, proteins, and DNA (Surai and Kochish 2019). This is followed by the second most important source of free radicals, phagocyte cells, which produce free radicals to kill pathogens. However, as some of these free radicals escape, they can impose damage to healthy tissues (Surai and Kochish 2019). Furthermore, transition metals (Fe2+ and Cu+), excess polyunsaturated fatty acids, and high oxygen concentrations are part of a wide range of external and internal factors that increase free radical production.

Evolutionally, multiple antioxidants defense systems have been developed and are responsible for the higher eukaryotes survival rates and can be summarized into three main lines. (1) detoxification of superoxidase radical through SOD (Surai 2016), which produces hydroperoxide (H2O2) that is toxic and must be removed from the cell by GSH-Px through conversion to water. Another essential element of the first line of defense is metal-binding proteins that are important to sequester free form transition metals which are involved in free radical formation. These metal-binding proteins are part of mitochondria integrity matrix. (2) chain breaking antioxidants that work in various mechanisms of antioxidants recycling. This includes but not limited to, vitamins E, C, carotenoids, GSH system, selenoproteins, and others. For example, vitamin E is usually oxidized after reacting with free radical and losses the antioxidant protective activity, but the presence of vitamin C helps converting vitamin E again to a rather "reduced" active form. Then, vitamin C is oxidized in turn but is further reduced by thioredoxin (TR). (3) heat shock proteins (HSP), methionine sulfoxide reductase, phospholipase, and DNA repair enzymes work together on preventing damage to biological molecules, lipids, proteins, and DNA.

As mono-gastric farm animals commercial production becomes more integrated, and production is more intensive, multiple stressful conditions require better management systems and regulating antioxidants defense systems in poultry via application of dietary supplements. To maintain poultry optimal productive and reproductive performance, feed rations must provide optimal dietary levels of vitamin E, Se, and carotenoids. Selenium, for example, expresses its unique antioxidant features through involvement in expression and synthesis of 25 selenoproteins, glutathione peroxidase (GSH-Px, TrxR, and SeP) (Sun et al. 2019; Zhao et al. 2017;

Li et al. 2018b; Surai and Kochish 2019). The SeCys from of Se works efficiently as a one main component of multiple selenoproteins that are essential modulators of a stronger antioxidant defense system through its effects on antioxidant enzymatic or non-enzymatic defense mechanisms.

The organic form is the main Se form that is supplied from grains with SeMet representing at least 50% of the total Se. With dietary concentrations vary greatly and most of the time is deficient, Se is incorporated in premixes across all poultry diets at 0.1–0.3 ppm. Dietary Se supplementation in poultry diet is in various forms including inorganic; selenite and selenate, and organic; Se-yeast, SeMet, OH-SeMet (Surai and Fisinin 2014). A majority of Se absorption (~80%) would occur in the small intestine, specifically in duodenum and jejunum regions. Absorption efficacy also varies among different Se forms with most absorbed for organic (SeMet) to medium for Selenate and poor absorption for Selenite. Passive absorption is considered selenite's main route while Na+ mediated absorption mechanisms are utilized by selenate and organic Se (SeMet) are absorbed the same way as pure methionine (Surai and Kochish 2019). After absorption, Se binds to blood proteins and is delivered to the liver which converts all forms of Se to hydrogen selenide (H2Se), an essential molecule for selenoprotein synthesis. Inside the cell selenoprotein expression and synthesis are regulated by Se status and stress level. Selenoproteins that are responsible for maintaining important cellular functions are known as housekeeping selenoproteins and those are usually not significantly affected by Se status of stress levels. Others that are stress responsive usually modulate their expression or synthesis by dietary Se supplementation and surrounding environmental conditions.

2.2.3.2 Mycotoxins

Mycotoxins, toxic metabolites that are produced by mold, yeast, and sometimes bacteria are considered another type "dietary" challenges, mostly presented directly from contaminated feed. These are defined as secondary metabolites of fungi that can cause serious health problems in animals, which would reflect on deleterious effects in farm animals ranging anywhere from lower feed consumption and growth impairment to lower diseases resistance to even death. Recently, fungi have been designated as a greater threat to animal, plant, and ecosystem health than the other taxonomic classes of pathogens (Fisher et al. 2012). The major problem associated with mycotoxins contaminated animal feed is not acute disease episodes, rather it is the ingestion of low levels of toxins over a longer period "chronic" which may cause an array of metabolic, physiologic, and immunologic disturbances (Oswald et al. 2005; Bryden 2012). A substantial progress has been made in mycotoxin research regarding effects on intestinal functions in recent years. By contrast to the limited distribution of mycotoxins into systemic tissues, the GIT is exposed to all the mycotoxins in contaminated feed. This suggests that the intestinal epithelium is the major site for the effects of mycotoxin contaminated material, even low levels of contamination. So, the constant exposure of the intestinal epithelium cells to 100% of contaminated feed is always cited as the first target of these contaminants. These epithelial cells can either be compromised by mycotoxins ingested prior to

absorption in the upper GIT region or throughout the entire intestine by non-absorbed toxins. Non-absorbed mycotoxins are constantly located in the lumen, they negatively affect the gut integrity and epithelial cell's structure and with most mycotoxins quickly appear in blood circulation, this clearly indicates that the proximal part of the GIT is the main site for absorbing majority of the ingested toxin (Cavret and Lecoeur 2006).

As of early 2000, many studies have focused creating in vitro models studying mycotoxins effects on intestinal permeability. Differentiated and polarized intestinal epithelial cells into a polarized monolayer became a particularly useful tool with TEER (Transepithelial electrical resistance) and are utilized as a good feasible indicator of the epithelial barrier integrity. TEER can be significantly reduced by exposure to different concentrations, types of mycotoxins, particularly DON. Tight junctions (TJ), a major component for the epithelial cells' integrity is formed from different proteins including ZO-1 and one or more claudin, and they seal the lumenal end of the intercellular space limiting transport of small hydrophilic molecules by this paracellular route. ZO-1 acts as a bridge organizing transmembrane TJ proteins and attracting signaling molecules to the complex, while claudin binds to ZO-1 and plays a key role in regulating permeability through TJ (McLaughlin et al. 2004). Therefore, in most of the published research studies the claudin protein family are referred to as a key determinant of paracellular permeability. Many studies showed that either by immunofluorescence or immunoblotting, the expression of claudin 4 on the intestinal epithelial cells was either reduced or even removed (McLaughlin et al. 2004; Lambert et al. 2007; van de Walle et al. 2010; Pinton et al. 2010; Diesing et al. 2011; Pinton et al. 2012) as animal/bird was fed DON-contaminated feed. Claudin 4 expression in the jejunum was reduced as pigs were fed lower DON concatenations for five consecutive weeks (Pinton et al. 2009). DON impose a negative effect on total protein synthesis (van de Walle et al. 2010), this was linked to reduce claudin 4 expression.

Fumonisin (FB) is another example, being poorly absorbed in the GIT of monogastric animals (1–6%) indicates that the epithelial layer is at great exposure to a higher toxin ingested. Similarly, the absorption of deoxynivalenol (DON) is considered moderate in pigs (55%) but fairly limited in poultry (5–20%). No data of FB1 on claudin is available, while occludin and ZO-1 show inconsistent results. However, FB1 has a key role in sphingolipids metabolism (Loiseau et al. 2007) which in turn have a significant role in the establishment and maintenance of TJ (Lambert et al. 2007). Therefore, GIT epithelium cells might be a target for FB1 negative effects. This was noted in lower expression of claudin and E-cadherin in the ileum of pigs as they were fed lower FB1 dosage (Bracarense et al. 2012).

Intestinal lesions due to mycotoxin exposure are always dominant in the duodenum and jejunum. Following the ingestion of DON-contaminated feed, proximal intestinal epithelial cells are exposed to high concentrations of DON. In fact, with as extraordinarily little DON being absorbed in poultry upper GIT it still acts as an inhibitor to protein synthesis at the ribosomal level, especially at the high proliferating cells in proximal GIT tissues that are characterized by high protein turnover, including immune system and intestine (Dänicke et al. 2002). Though, it is

not surprising to see significant reduction of duodenal villus height, for example, which is a clear demonstration of DON alteration to mucosal structural following toxin ingestion. The effectiveness of nutrients absorption would be compromised due to reduced villus height (Grenier and Applegate 2013). During the migration along the crypt-villus axis, enterocytes must differentiate to fully express their digestive functions indicated by an increase in enzyme activities including sucrase and maltase (Applegate et al. 2009). However, feeding DON-contaminated feed would always be associated with shorter villus and impaired nutrient absorption because of a smaller number of differentiated epithelial cells (Grenier and Applegate 2013). Another example of DON assault to proximal intestinal cells is through modulation of intestinal paracellular transport which leads to an increased passage of macromolecules and bacteria (Pinton et al. 2009). As a result, the intestinal barrier integrity and function would be negatively affected leading to increased permeability or "leaky gut." The reduction in epithelial integrity will then contribute to an increased protein availability in the intestinal lumen due to plasma proteins and amino acids leakage into the gut, which creates a favorable environment of massive overgrowth of pathogens including C. perfringens. Indeed, Antonissen et al. reported an increase in duodenal total protein levels (Antonissen et al. 2014). Nutrient's malabsorption could be the reason, due to the negative effect of DON on nutrients digestion or increased plasma amino acids and proteins leakage into the intestine because of altered intestinal barrier integrity. Reduced duodenal villus height has also been reported to be associated with nutrients maldigestion and malabsorption. Multiple studies have shown different intestinal transporter proteins for different nutrients being selectively modulated by DON. This would negatively affect sodium associated amino acids co-transport of serine and proline resulting in an increased lumenal contents of such amino acids (Awad et al. 2004; Dietrich et al. 2012; Huang et al. 2015; Bedford and Gong 2018; Upadhaya et al. 2020; Xiong et al. 2019).

2.2.3.3 Infectious Agents

Infectious bacteria or viruses are a major threat for GIT barrier function and often directly target intestinal epithelial cells. Some pathogens thrive within these cells (such as *Lawsonia intracellularis* in swine) and attempt to subvert the immune system to avoid their destruction (Vannucci and Gebhart 2014). This is also an apparent strategy of parasites such as Eimeria spp., the causative agent of cocciodioses, which induces production of anti-inflammatory IL-10 in the host to subvert immune responses (Abdul Rasheed et al. 2020). Additives like probiotics or microbial components could act as immune stimulators, which could circumvent these immune-invasion strategies (Ohashi and USHIDA 2009; Tiwari et al. 2020). However, when no pathogens are encountered during the life cycle, chronic stimulation of immunity may come at a metabolic cost or cause low-grade chronic inflammation (Arsenault et al. 2017).

Another strategy to prevent GIT barrier damage due to infectious agents would be to prevent infections altogether. Some probiotics affect viability of pathogens or achieve this indirectly by promoting a healthy microflora. Other additives, such as butyric acid or medium-chain fatty acids, may affect virulence of pathogens such as

Salmonella spp. (van Immerseel et al. 2006) or may reduce viability of certain viruses (Thormar et al. 1987). Some supplements have been claimed to block access of pathogens to epithelial cells by interfering with pathogen-binding to host cells (Fernandez et al. 2000). Access to epithelial cells could further be restricted by increasing mucin production, which can be increased by butyrate (Gaudier et al. 2004; Sikandar et al. 2017). This short-chain fatty acid is often added to the diet or it is raised by increasing the number of butyrate-producing bacteria in the gut, for example by adding certain prebiotics or probiotics. Pre-biotics can also be produced through enzymatic breakdown of NSP (Non-Starch Polysaccharides) (Yacoubi et al. 2017), possibly even within the gut from plant NSP upon exogenous enzyme administration. Mucin production can also be enhanced with other supplements, such as amino acids (Zhang et al. 2019; Dong et al. 2017). Host-defense peptides produced by epithelial cells may further limit the viability of infectious agents, and their production can also be stimulated with butyrate (Zeng et al. 2013) or probiotics (Wang et al. 2019) and possibly other supplements. The effect of butyrate likely also extends to stimulation of host-defense peptide production by intestinal macrophages (Sunkara et al. 2012; Schulthess et al. 2019).

If infection cannot be prevented, it is then essential to at least limit harmful effects of collateral damage which occurs mainly in two ways: Inflammation and oxidative stress. Unless a pathogen manages to subvert the host cell, the infected cell will send alarm signals to attract immune cells. These signals typically elicit an inflammatory response which helps clear the pathogen, but it may also impair gut barrier integrity by weakening TJ function. Many feed additives can effectively exert anti-inflammatory effects on intestinal epithelial cells and conceivably also nearby immune cells. Examples are several strains of Bacillus that can dampen inflammatory signaling in epithelial cells by interfering with NFkB signaling (Rhayat et al. 2019) or in macrophages by inducing an M2 phenotype in these cells, which is anti-inflammatory (Paynich et al. 2017). Butyrate also exerts anti-inflammatory effects in the gut (Segain et al. 2000; Jiang et al. 2015; Wang et al. 2018) although this is likely dose- and location dependent.

A second consequence of infection occurs mainly when phagocytes are newly recruited into the gut and help clear invading pathogens. These cells (mainly macrophages and neutrophils, or “heterophils” in birds) have not yet adopted the tolerant phenotype and besides significant amounts of pro-inflammatory cytokines often produce reactive nitrogen species (mainly Nitric Oxide) or superoxide radicals to kill the invaders. Excessive production of these oxidants can further flame inflammatory responses and may directly damage intestinal tissue (Kruidenier et al. 2003) or even weaken tight junctions in epithelial cells (Rao 2008). Oxidative stress responses can also be provoked in epithelial cells themselves, for example when they are exposed to mycotoxins (Del Regno et al. 2015; Mahfoud et al. 2002). Supplementation of poultry or swine feed with Tocopherols (vitamin E) and Selenium may reduce oxidative stress responses in challenged conditions. Tocopherol scavenges reactive oxygen species (ROS) while Selenium gets incorporated into the catalytic unit of enzymes (as selenocysteine), thereby enhancing the removal of ROS. Together, both supplements can help maintain intestinal barrier function and

reduce oxidative stress in swine experiencing heat stress (Liu et al. 2016). Plant-derived polyphenols may also help reduce oxidative stress in the intestine and may thereby stop the vicious cycle that is sustained by a leaky gut (Gessner et al. 2017; Prakash and Srinivasan 2010).

As much as a healthy gut microbiome plays a key role in maintenance of GIT barrier function, among others by controlling pathogens, strengthening immunity and providing functional and nutritional metabolites (e.g. butyrate), an unhealthy microbiome can have a very detrimental effect. It is generally accepted that a healthy microbiome is characterized by a strong diversity in its composition at every level (from domain level via phylum to species and strains). In some cases, however, this balance is disturbed leading to a flourish of a limited set of species that will overwhelm the host-microbiome equilibrium. This state of "dysbiosis," which is still poorly defined, may lead to massive production of harmful metabolites and toxins which can cause significant breakdown of gut barrier function. Dysbiosis can be caused by poorly digestible diets (Ducatelle et al. 2018; Gresse et al. 2017) or by antibiotics treatments (Guevarra et al. 2019) and can be reversed by several additives, including pre- and probiotics (McFarland 2014; Ducatelle et al. 2015).

Some dietary factors not only threaten GIT barrier function by inducing microbial dysbiosis. Certain anti-nutritional factors commonly present in many food staples may cause intestinal epithelial damage, such as soybean agglutinins (Zhao et al. 2011), while others such as soy galactomannans may indirectly damage the barrier by activating intestinal immune cells (Arsenault et al. 2017; Pont et al. 2020). Human and rodent studies had shown that high-fat, high energy diets cause a breakdown of gut barrier function with translocation of LPS into the circulation in the postprandial state (Cani et al. 2007). This is problematic for animal production because current chicken and swine breeds ingest incredible amounts of food and are almost permanently in a postprandial state. While reduction of feed intake thus is not an option, it is clear that solutions are needed to prevent GIT barrier breakdown and maintain health and performance.

2.3 Concluding Remarks

The GIT is literally at the center of health and well-being and disturbances in GIT barrier function are either a cause or consequence of a variety of disorders. Therefore, nutritional support of GIT barrier function may be a promising strategy to support health and growth of modern production animals.

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