Chapter

# Secretory Defense Response in the Bird's Gastro-Intestinal Tract and Nutritional Strategies to Modulate It

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## Abstract

The Gastrointestinal tract (GIT) is a very complex environment which converges a lot of players including nutrients, microorganisms, pathogens, cells, and peptides which determine the type of outcome against threats affecting feed efficiency and body weight gain. Traditionally, GIT is examined as a selective barrier which permit or deny the entrance of molecules, but beyond of that, it is a crucial place to produce important proteins for the host which, at least in part, determine the outcome for a threat such as microorganisms, toxins, anti-nutritional factors, among others. During the non-antibiotic promotants time, there is a necessity to understand how this system works and how we can modulate through nutrition, in part to take advantage of this, and support a better immune response and nutrient absorption in challenged poultry environments. The goal of this chapter is to review the different mechanism of immunity in the GIT emphasizing on secretory defense response and the nutritional strategies including fiber and fatty acids to improve it.

**Keywords:** antibiotic growth promotants, mucins, M cells, paneth cells, goblet cells, host defense peptides, trefoil factors, IgA, dietary fiber short chain fatty acids, medium chain fatty acids

## 1. Introduction

There is a tremendous interest in the understanding of immune response against pathogens and toxins on the gastrointestinal tract (GIT) of the birds due to in this specialized system, as it harbors 70 to 80 percent of the avian immune cells and molecules [1]. Additionally, there is an overwhelming interest in finding new alternatives to antibiotic growth promoters (AGP) because of regulations and consumer preference in many countries which these strategies are banned or regulated. The mucosal surface of the GIT is covered by a monolayer of columnar epithelial cells. This epithelium represents a vast surface that is vulnerable to foreign immunogens (i.e. food-borne antigens), microbial pathogens and toxins. By being in contact with a large number of potentially harmful substances and infectious organisms, the mucosal surface must provide a means to not only regulate active and passive absorption of macromolecules but also provide as a general and selective defenses

in part through secretory antibodies and other mucosal defense mechanisms. Consistent with these functions, the epithelial surface of the GIT is lubricated and protected by mucus secretion and by a highly specialized immune system underlying the epithelium which exports immunoglobulins into the intestinal mucosa. Secretory defenses are some of the most important means to protect the intestinal epithelium from enteric pathogens and toxins. Secretory IgA (sIgA) production, Goblet, Paneth, M cells and GALT tissues are the key cells in this defense. The objective of this review is to describe a variety of secretory immune responses against pathogens in GIT and the role of nutrients in immunomodulation.

## 2. Histology and physiology of the gastro-intestinal tract

Gallus species have villi which decrease in length from 1.5 mm in the duodenum to 0.4–0.6 mm in the ileum and rectum. The number of villi decreases from 1 to 10 days of age, but thereafter remains constant. Genetic selection for growth has altered villi morphology [2]. The villi of broilers are larger than White Leghorns, and show more epithelial cell protrusions from the apical surface of the duodenal villi. However, the villi from both types of chickens consist of a zig-zag arrangement which is thought to slow the passage rate. The intestinal wall contains four layers as including the mucosal, submucosal, muscle tunic, and the serosal layer. The mucosal layer consists of the muscularis mucosa, lamina propria, and epithelium. However, the muscularis mucosa and lamina propria are poorly developed in chickens, possibly because of the absence of a central lacteal. Although Brunner's glands, common to mammals, are absent [3] tubular glands possibly homologous to Brunner's glands, are present in some birds [4]. The epithelium has chief cells, goblet cells, and endocrine cells. The crypts of Lieberkühn are the source of epithelial cells lining the villi. The crypts contain undifferentiated cells, goblet cells, endocrine cells, and lymphocytes. Globular leukocytes and Paneth cells appear near the base of the crypts. The intestine contains extensive innervation from both the sympathetic and parasympathetic nervous system. As described [5], innervation is both cholinergic and adrenergic. Contraction of the rectum appears to be mediated by noncholinergic, non-adrenergic nerves [6, 7].

The mucosa of the GIT is a functional interface between the environment and the internal physiological compartments of the organism. As such, the mucosal and associated cells constitute a dynamic and metabolically active barrier possessing selective permeability [8]. This barrier has multiple functions that involve the digestion, transport and uptake of specific substances and nutrients and exclusion of microorganisms and toxins. The processes of digestion and absorption occur in a micro-environment modified by the intestinal mucosa, its secretions, and the ancillary organs (pancreas, liver). The importance of 'the intestinal barrier' as it relates to gut function and gut health in poultry has been reviewed [9, 10]. Optimal digestive and absorptive functions are essential for growth, development and health of the animal. In addition, the intestine must act as a physical barrier to pathogenic organisms and toxins and play a role in both innate and acquired immunity. The integration of the digestive, absorptive and immune function of the GIT and the genetic regulation of these processes are central to animal production and health.

## 3. Innate immunity of the GIT

The epithelial cell physical barrier in the GIT represents a vast surface area that is very vulnerable to intraluminal impacts. Continual confrontation by direct

contact with foreign substances, the mucosal system is tightly regulated in order to allow selective entry of macromolecules necessary for mucosal defense [11]. The cells and molecules that comprise the innate immune responses encompass both physical and chemical barrier mechanisms. For example, epithelial cells are tightly connected by multi-protein junctional complexes which regulate passage of solutes while providing an obstacle to luminal microbes and the lamina propria. Mucosal epithelial cells also produce non-specific macro-molecules (such as defensins) with antimicrobial action. Inflammatory and anti-viral responses are produced by specific mucosal cell types, which include: dendritic cells (DC), macrophages, and innate lymphoid cells (ILC). Pattern recognition receptors on these cells regulate many of these responses through interaction with microbial ligands [12].

### 3.1 Mucus and mucins

The intestine is protected by that substance, which forms a tightly adherent layer along the epithelial surface, followed by a more loosely adherent, partially hydrolyzed layer. It is also part of an integral process, and is secreted, forming and "unstirred" water gel layer covering the epithelial surface. This gelatinous molecular "coat" is subjected to continuous erosion by luminal fluid flow and rapid replenishment from epithelial secretion. The dynamics of mucus gel turnover contributes to a complex milieu where digestive events occur, nutrients approach epithelial cells, microbes build ecological niches, exfoliated enterocytes break down and immunological molecules (defensins, IgA, etc.) carry out surveillance. Consequently, the mucin layer, which encompasses all the of these components, constructs a gel-like biological barrier that shields the underlying tissue compartments, and eventually serves as an important component of the innate arm of the host system in the GIT [11]. In the small intestine the mucus layer is penetrable, but the bacteria are kept away from the epithelium by antibacterial mediators. In the large intestine, the inner mucus layer is impenetrable to bacteria whereas the outer mucus layer is expanded and serves as the habitat for bacteria (esp. mucolytic bacteria) [13]. Serving not only as a lubricant but also a protective barrier, the mucus gel layer(s) in the GIT is the largest area and of critical importance to the body both physiologically and nutritionally. Compromised mucin function is associated with many gastro-enteric disorders and nutritional insufficiencies. Particularly, many functional modulations of the GIT are closely related to expressional, structural, and physiological alterations of mucus and its major components [14].

The protective functions of mucus are attributable to mucus glycoproteins, the major macromolecules present in the mucus gel. Mucus glycoproteins, now widely known as mucins, are defined as a class of high-molecular-weight proteins that are heavily glycosylated with complex oligosaccharide chains [15]. The molecular weight of mucins has been estimated from early studies of ~1000 kDa with attached carbohydrates accounting for 80% of the mass [16].

According to cellular localization and distribution, mucins are broadly classified into secretory and membrane-associated proteins [17]. Structurally mucins are comprised of a linear protein backbone in the center and a large number of carbohydrate chains attached around it. The carbohydrate components, usually heterosaccharides, are bound covalently to the peptide chains and terminated with sialic acid (sialoglycoproteins) or with both sialic acid and sulphate ester (sialosulphoglycoproteins) or with neutral ends (neutral glycoproteins). These ends determine the extent of negative charges on each mucin molecule [17].

Intestinal secretory mucins are synthesized and secreted by goblet cells, a specialized wine-goblet-shaped epithelial cell lineage dispersed along the intestinal lining. The dimerization and/or polymerization of mucin molecules and the

electrochemical properties of mucopolysaccharides are believed to determine the chemical and biophysical characteristics of mucus along the GIT [18].

Mucins have a key role in avoiding potential damage from microbes. The mechanism by which mucus controls microflora colonization is referred to as part of innate epithelial cells [19]. The role of mucin on microbe colonization is manifested in at least two distinct ways. First, some microbes are mucolytic, including Bacteroidetes, and use mucin glycoproteins and carbohydrates as an energy source and provide physical support for intestinal colonization. Moreover, these bacteria provide substrates for other bacteria in the outer mucus layer by degrading the mucins [20, 21]. Second, mucins are generally "toxic" to the proliferation of certain microbes. Mucus gel inhibits proliferation by entrapping microbes that are starved or killed by antimicrobial peptides, and/or expulsed by the luminal flow. Mucus also provides a physicochemical barrier to prevent microbes from direct contact with epithelial cells.

Moreover, the mucus gel provides a matrix for antimicrobial molecules, which are mainly produced by Paneth cells. Direct interactions with mucins can facilitate the diffusion of these antimicrobial molecules [22]. Taken together, mucins have been proposed to play an important role in shaping microbial communities at the intestinal mucosa. Recent studies suggest the correlation between changes in mucin glycosylation profile and deviations of overall microbial community ecology as well as altered abundances of specific microbes [23, 24].

### 3.2 Trefoil factors

Co-expressed with mucin-secreting cells and in close relation with mucus, trefoil factors (TFF) demonstrate an interesting group of mucus molecules. Trefoil factors were initially discovered in the pig pancreas [25] and further characterization of this family has strikingly observed their abundant expression in the GIT and their efficacy as therapeutics especially for preventing and treating various GIT conditions [26, 27]. They are named as trefoil by their "three-leaf" structure and are a family of small (7-12 kDa in mammals) protease resistant peptides whose common unit is the trefoil motif [25].

It is now clear that TFF participate in the healing of mucosal injury in disease conditions by promoting cell migration over damaged areas (rather than promoting cell division), and inhibiting cell death, and are also believed to be involved in physiological repair of epithelia from daily apical sloughing against frequent luminal insults [25, 28, 29].

TFF have recently been found to participate in immune responses. It was showed that TFF2 deficiency or administration of recombinant TFF2 altered the expression of immune associated genes including defensin genes in Paneth cells [30]. The presence of TFF in immune organs, including spleen, thymus, lymph nodes and bone marrow [31], may suggest possible regulatory role(s) played there. TFF can be a potent mitogen by regulating chemotaxis, stimulating the migration of immune cells. The molecular basis of such may be supported by the recent in vitro evidence that recombinant TFF2 activates CXCR4 chemokine receptors and attenuates CXCR4 mediated chemotaxis [32]. This finding also highlights a molecular linkage between TFF and the immune system.

TFF are thought to cooperatively interact with mucins in the lumen to enhance the protective barrier properties of the adherent mucus layer against bacterial and toxic insults [25, 28]. Thim et al. [33] observed significant increase in the viscosity and elasticity of gastric mucin solutions because of TFF2 addition [33]. Increased viscosity could help prevent antigens from approaching the epithelium surface, especially in healing epithelia, which eventually benefits epithelium restitution and

alleviates immune system burden. In this scenario, TFF are predicted to be involved in mucus polymerization.

### 3.3 Goblet cells

Goblet cells together with absorptive enterocytes, Paneth cells (secreting antimicrobial peptides etc.) and enteroendocrine cells, represent the four principal cell types that are continuously renewed in the epithelium of the small intestine. During intestinal epithelial cell regeneration, pluripotent stem cells that reside at the bottom of the crypt divide to generate multiple cell lineages which migrate from the proliferative crypts to the villus tip [34]. While migrating along the crypt-to-villus axis, goblet cells are terminally differentiated from secretory cell lineage derived from a common Math1-expresing progenitor cell [35]. Goblet cell differentiation is controlled by winged helix transcription factors Foxa1/a2 which can also transactivate Muc2 promoters [36].

It is generally believed that goblet cells producing neutral mucins contain little sialic acid and represent an immature state; while goblet cells containing acidic mucins are more likely resistant to infections because they are normally "upregulated" in response to bacterial infection. In addition to mucins, several other molecules are co-expressed within the intestine such as ingobsin (localized in human and rat goblet cells) with endoproteolytic activity in the presence of both epidermal growth factor and cobalamin-binding protein haptocorrin [37]. TFFs are (specifically TFF3) along with mucins biomarkers of goblet cells.

### 3.4 M cells

M cells or Microfold cells (because of uneven microvilli) are classified as epithelial cells with large fenestrations in their membranes. These features enhancing the uptake of antigens from the gut lumen [38]. They have a capability for capturing luminal antigens and transporting them across the epithelium ("transcytosis"). They are placed in the gut epithelium called follicle associated epithelium overlying the domes of Peyer's patches and other lymphoid organs. M cells are not professional antigen-presenting cells because they do not have the ability to process and present antigens to the major histocompatibility complex (MHC) molecules. Instead, they serve as antigen delivery cells, that is, as a functional equivalent to lymphoid nodes because they provide antigens to professional antigen-presenting cells, such as dendritic cells (DCs), macrophages as well as B lymphocytes. Indeed, many pathogens take advantage of their transport efficacy to invade the body [39–41]. M cells subsequently transfer these antigens to underlying DCs enabling the transfer of captured molecules through transcytosis mechanism (which remain to be elucidated) as well as intracellular material through microvesicles to underlying DCs [42]. In conclusion, M cells provide specialized full-service immune surveillance capabilities.

### 3.5 Paneth cells

Paneth cells are physiologically found at the distal small intestinal crypts of Lieberkühn and contain abundant secretory granules. Their unique histomorphological features implicate special functions in cellular homeostasis as well as in the establishment and configuration of the mucosal barrier as a physical and highly organized immune interface [43]. Previous studies suggesting the existence of Paneth cells in the chicken remained controversial. However, recent research has supported Paneth cells existence in the small intestine of the chicken by electron

microscopy confirming the presence of granulated secretory cells at the base of the crypts in the chicken small intestine. The researchers also confirmed by Western blot the expression of lysozyme protein, which is specifically secreted by the Paneth cells in the small intestine [44]. Paneth cells have the morphological characteristics of a professional secretory cells, including an extensive ER, a Golgi apparatus and an internal secretory granule. The first assumption that Paneth cells had a host-defense function emerged when lysozyme was identified as a product of these cells [45]. After that, it was discovered that Paneth cells secrete antimicrobial peptides (AMP) or host defense peptides (HDPs) which are important host-defense substances in the communication between host and microbiome. One of the most well characterized are β-defensins [46]. In addition to defensins, Paneth cells is able to secrete other AMPs including secretory phospholipase A2, Reg III, angiogenin 4 and cathelicidins [47–49].

### 3.6 Host defense peptides

HDPs are generally positively charged small peptides with amphipathic properties [50]. These peptides present in the GIT display an important, but often overlooked role in the first line of defense. With the first avian HDPs identified in 1990s [51], the information about avian HDPs has increased considerably in the subsequent decades. Currently, avian β-defensins and cathelicidins are the two major classes identified and extensively studied in chickens [52, 53].

HDPs were initially called antimicrobial peptides (AMPs), because they are characterized by the direct antimicrobial activities against a broad spectrum of numerous pathogens, including gram negative and positive bacteria, fungi, and even certain viruses [54–56]. Generally, the cytoplasmic membrane of pathogenic organisms is a frequent target for HDPs. The amphipathicity and cationic charge of HDPs allow the initial contact with membrane electrostatically, as most bacterial surfaces are hydrophobic and anionic. The peptides then insert into phospholipid bilayers and induce pore formation in membranes by toroidal pore formation, carpet formation and barrel-stave formation, resulting the cytoplasmic leakage and death of pathogens [54, 57–59]. Besides pore formation in membranes, some HDPs can directly penetrate into cells and interfere with intracellular molecules, interrupting cell wall formation, DNA and RNA synthesis, protein translation and post-translational modification [57, 60].

To be specific, chicken AvBD1, −2, and − 7 exhibit high efficiency against a large variety of both gram-negative (*E. coli*, S. enteritidis, S. typhimurium, C. jejuni, and *K. pneumoniae*) and gram-positive (*S. aureus*, B cereus, *L. monocytogenes*, S. haemolyticus, and S. saprophytus) bacteria [51, 61–64]. AvBD1 and − 7 also efficiently kill P. aeroginosa and E. cloaca, while AvDB2 showed reduced efficacy [61, 64]. AvBD4, −5, and − 11 protect host from invasion of S. enteritidis and S. typhimurium, however their antimicrobial activities on other bacteria species remain to be determined [63, 65, 66]. Although AvDB8, −9 and − 13 are active against *E. coli*, respectively, they exhibit a minimal activity against several other bacteria [66–69]. Based on studies of different AvBD isoforms, it seems that both structure and catholicity are important for antimicrobial activity but disparity in the preference of gram-negative or positive bacteria.

All four chicken CATHs show antimicrobial capacities in the same order of magnitude against a wide range of gram-negative and positive bacteria, and fungi [70–73]. Similar to AvBDs, the structure and cationic charge are equally important for their antimicrobial activities. The presence of an alpha-helical region in N-terminal and hinge region around the center of the peptide are important for antimicrobial. Removal of N-terminal alpha-helix in CATH2 truncation or

disrupted helix formation in a-helical synthetic peptide leads to the loss of antimicrobial activity [72, 74, 75]. Although deletion of C-terminal alpha-helix in CATH2 reduces the activity against pathogens, the remaining truncation is still capable to kill bacteria [75]. The truncation of CATH2 with N-terminal alpha-helix alone shows increased antibacterial activity [76]. The hinge region plays a key role in the insertion of CATH into the bacterial membrane and pore formation [74, 77]. Disruption of the hinge region by point mutation or removal in the center of the CATHs largely decreases the antimicrobial activity [72, 74, 78]. The cationicity of CATH and AvBDs is important for the initial contact with the surface of bacteria. The higher cationic charge in CATH2 and the synthetic analogs results in the better antimicrobial outcomes [72, 75].

In addition to direct antimicrobial activity, the HDPs exhibit the immunomodulatory function, involving inflammation and chemotaxis. Chicken AvBD13 was reported as a direct TLR4 ligand [79], increases production of IFN-γ and IL-12 in mouse monocytes through activation of TLR4-NFκB axis. Combined with the evidence that AvBD13 increases serum IgG and IgM levels in chicken and induces lymphocytes proliferation in spleen after the administration of the infectious bursal disease vaccine (IBDV) [80], activation of TLR signaling by AvBD13 indicates an immune enhancement rather than a merely pro-inflammatory effect. Moreover, chicken AvBD1 fusion protein expressed by IBDV enhances CD4+, CD8+, and CD3+ T-cell proliferation, increases antibody titers, improves survival rate in in vivo experiment [81]. Additionally, HDPs have been shown chemotactic effect. Investigations about immunomodulation by avian AvBDs and CATHs are mainly limited to NF-κB activation, cytokine production, and direct immune activation. The similar findings in human and mouse studies suggest the conserved function of HDPs among species, providing the guideline for the application and future research in poultry area.

## 4. Adaptative immunity of the GIT

Unlike the innate immune system which attacks only general threats, adaptative mucosal immune system is triggered by exposure of potentially dangerous pathogens. However, sometimes if overlaps some of their functions. The three most key roles of that system are: the induction of an efficient and appropriate immune response to pathogenic invaders, the tolerance of the commensal microorganisms of the intestine as well as the induction of the tolerance of nutrients and other environmental immunogens. Responses of the systemic immune system can originate from or be modified by the mucosa; this is exemplified by the attenuation of systemic immune responses to a protein that has first been fed orally to the animal (oral tolerance). Thus, the mucosal immune system must maintain the delicate balance between responsiveness to pathogens and tolerance to a vast array of other harmless antigens encountered at mucosal sites. This balance is achieved through the interplay of innate and adaptive (B- and T-lymphocyte) mechanisms [82].

The adaptative immune system in the GIT has features that are distinct from adaptative immune systems in other organs. The major form of adaptative immunity in the gut is humoral immunity directed at microbes in the lumen. This function is mediated mostly by dimeric IgA antibodies that are secreted into the lumen of the gut. Cellular adaptative immunity is carried out by an intraepithelial lymphocytes (IEL) in healthy adult bird includes major subsets of NK and T cells bearing the γδ or αβ form of the T cell receptor (TCR). In contrast to other tissues, B cells are almost entirely absent from the IEL and the T cells predominantly express the CD8 co-receptor with smaller populations of TCRαβ+ CD4+ and CD4 + CD8+ cells [83, 84].

Moreover, within the CD8+ IEL population the majority express CD8αα homodimers rather than the CD8αβ heterodimer commonly expressed on classical CD8+ T cells found at systemic sites [83–85]. The proportions of IEL belonging to each subpopulation differ according to age, genetics and environment (including infection). Numerically, B and T cells are the most common lymphocytes (~90%), the remainder being of the NK cell phenotype (CD3-Bu-1-). In contrast to the IEL population, the T cell population of the lamina propria contains a smaller proportion of γδ-T cells (~10%); the much larger αβ-T cell population is dominated by CD4+ T cells, with a less prominent CD8+ cell population [86].

### 4.1 Secretory IgA (sIgA) and its transporter, polymeric Ig receptor (pIgR)

The existence of sIgA in the bird has been established for quite some time, but studies are relatively limited compared with mammals. In humans, it is estimated that approximately 70% of the body's IgA-producing plasma cells (differentiated from activated B cells) reside in the lamina propria of intestinal mucosa [87–89]. Although sIgA belongs to adaptive immunity by definition, it plays an important role in the first lines of mucosal defense [87, 90]. There are three modes of defense modulated by sIgA on gut mucosal surfaces: (1) sIgA has been shown to interfere with the early steps of the infection process through directly blocking pathogens and toxins from attaching to the intestinal epithelium [91]; (2) sIgA exerts the protective immunity through immune exclusion, which is the prevention of pathogens and toxins from approaching to epithelium through the stepwise procedures involving antibody-mediated agglutination, entrapment in mucus, and clearance through intestine peristalsis [92, 93]; (3) sIgA exhibits the ability to neutralize intracellular pathogens, viruses, and toxins within intestinal epithelial cells, which requires binding of specific IgA and occupation of antigens by pIgR transportation vesicles, followed by the passage of antigens into the lumen. Notably, the intracellular neutralization of LPS by IgA indicates the potential role in anti-inflammation and deactivation of the proinflammatory pathways in epithelial cells [90, 94, 95].

T-cells generally produce high-affinity IgA antibodies. IgA has the specificity against previous exposure of the GIT by pathogens and more invasive commensal species [89, 96]. Conversely, low-affinity IgA antibodies can also be produced from T-cell-independent (TI) pathways. These low-affinity IgA antibodies act through coating commensal bacteria thereby augmenting the competitive inhibition of pathogens [88, 89, 96]. The production of both high and low-affinity production of IgA provides protective roles during an overt infection with a pathogen as well as during unchallenged/non-pathogenic bacterial exposure.

Presence of microflora in the the GIT may also regulate production of IgA. Studies with germ-free mice [99] and pigs [100, 101] have demonstrated that intestinal IgA and IgA-positive cells in the lamina propria are dramatically reduced versus conventionally reared animals. Further studies have shown that specific microflora (e.g. segmented filamentous bacteria and clostridia) when given to germ-free mice will stimulate the development of IgA-producing cells, while other microflora will have no effect or inhibit this development [97, 98]. Thus, other researchers have reported similar IgA production responses with poultry diets were supplemented with probiotics [99, 100]. Notably, IgA development in the hindgut of the bird early in life coincides with the rapidity of bacterial colonization [101].

Prior to development of IgA by the GIT, the chick is reliant upon maternal antibodies and physical defenses (such as mucins and intestinal turnover). In birds, a small amount of IgA (~ 0.3 mg) is transferred via the embryo imbibing amniotic

fluid prior to internal pipping [102, 103]. The endogenous IgA expression starts to increase after the second week post-hatch [101]. Bar-Shira et al. [103] suggested that the resistance of rapid depletion of maternal IgA may be due to unique uptake by goblet cells, which serves as a reservoir to slowly release maternal IgA.

Circulating IgA is predominantly in a monomeric form, whereas in intestinal secretions it is found in a dimeric configuration both in mammals and birds [96, 104]. IgA secreted by plasma cells accumulates in the lamina propria. To exert its protective effect, pIgR is constitutively expressed by epithelial cells to transport IgA through the epithelia from the lamina propria to intestinal lumen. During the transcytosis, IgA is bound by pIgR on the basolateral surface and transported to the apical surface. At this surface, cleavage of the extracellular portion of pIgR results in release of secretory component (SC) as part of the dimeric IgA, otherwise known as the sIgA complex [105]. In this complex, sIgA is thought to be protected against degradation by proteases and pH fluctuations in the gut [90]. Excess production of pIgR which is not utilized as an IgA chaperone is also secreted as "free SC", which may have additional bacterial scavenger properties [106]. Once secreted, the N-glycans of SC can then bind to itself, and/or sIgA in the mucin layer thereby bridging these luminal defenses [105].

As one molecule of pIgR is required to bind and transport one dimeric IgA for secretion of sIgA into the intestinal lumen, pIgR's expression regulates sIgA capacity into the GIT [105, 107]. Expression regulation in mammals can be induced by numerous cytokines, including: interferon-γ (IFN-γ), tumor necrosis factor (TNF), interleukin-1β (IL-1β), and IL-4. These cytokines act through mediating a transcriptional response through activation of several transcription factor-binding sites in regulatory regions [105, 107–109]. In the chick, increases in IFN-γ, IL-1β and IL-4 expression in the second week post-hatch [110, 111] may influence subsequent increases in expression of the chicken pIgR gene [111]. Additional bacterial binding to Toll-like receptors have also been shown to increase pIgR expression in epithelial cells [105, 107, 112].

## 5. Nutrition and secretory immune response

The GIT is an extremely expensive tissue in terms of energy and nutrient needs to maintain and facilitate the full range of barrier and energy/nutrient assimilation functions it displays. Cant et al. [113] estimated that the GIT consumes approximately 20% of dietary energy with a turn-over rate of 50 to 75% per day. However, the GIT is a dynamic organ system whose maintenance needs dramatically changes based on responsive demands. Applegate [114] elucidated some of these adaptive responses, including: changes to peristaltic rate, changes to enterocyte turnover, tight junction regulation, mucin production (quantity and composition), changes to differentiation direction of undifferentiated cells and changes to secretory defenses.

While we often think of presence of microbiota as an additional barrier cost, there is some symbiotic relationships that they convey to the host. For example, the presence of the ceca contributes approximately 3% to dry matter digestibility to the bird [115] in part through 8% of energy derived from microbial fermentation resulting in short-chain fatty acids [116, 117].

Due to limitations of space in this review, we were unable to address all nutrient impacts on the secretory immune defenses of the bird. Notably, recent reviews have published on roles of amino acids on physiological, immunological, and microbiological responses as well as quantification of changes to endogenous amino acid production in the bird [118, 119]; as well as implications of protein indigestibility in

the GIT and implications of microbial fermentation of protein in the hindgut of the animal [120]. Additional impact of microminerals (e.g. zinc, copper, and manganese) and plant bioactive compounds on intestinal functionality have been elucidated [121, 122]. Similarly, recent research has revealed modes of action of specific classes of feed additives that directly or indirectly influence the secretory immune responses of the GIT. For example, probiotic and phytogenic additives have had numerous reviews on these actions [123–125]. Further elucidation of contribution of specific fibrous and fatty acids to the intestinal secretory defenses are further elucidated.

### 5.1 Dietary fiber and intestinal health

Carbohydrates that are not hydrolyzed by endogenous enzymes in the upper GIT can be fermented by bacteria in the large intestine and ceca are designated as dietary fiber [126]. Dietary fiber (DF) resides in the indigestible portion of plant derived foods that include cell walls, non-starch polysaccharides (NSP), oligosaccharides and lignin [126, 127].

Polysaccharides of NSP include cellulose, pectins, β-glucans, pentosans, heteroxylans and xyloglucan [128]. There are two different types of NSP, soluble and insoluble. Such classification is based on their solubility in water. The ability of soluble NSP to mix with water, producing an increase in the viscosity of the digesta and decreasing the binding of digestive enzymes, negatively affects the digestibility of nutrients [129]. As a result of suboptimal digestion, there is an increase in GIT surface area and secretion of digestive enzymes, creating an increased endogenous energy cost of digestion and affecting bird productivity [130]. NSP from cereal-based diets are associated with low apparently metabolized energy, increased feed conversion rates and increased incidence of wet droppings.

Some previous studies have considered the effects of different cereal NSP based diets on the intestinal microbial immunity. Different types of cereal can modify specific members of the microbiota in the cecum of chickens in two different ways; by altering the viscosity and pH and/or by supplying nutrients to produce the selective growth of specific bacteria [131]. The increase in digesta viscosity with the subsequent reduction in feed passage rate leaves more undigestible feed in the intestine, which represents an ideal substrate for bacterial growth [131]. Chickens fed with a barley-based diet had a higher number of *Clostridium perfringens* in the ileum and ceca. Likewise, it has been reported that the use of wheat in poultry diets may favor the proliferation of pathogenic bacteria like *Escherichia coli*, Salmonella and Campylobacter [132].

In contrast, insoluble NSP is metabolized into short chain fatty acids (SCFA) including acetate, propionate, butyrate, valerate and isovalerate [116]. Those fermentable metabolites serve as sources of carbon and energy for the commensal microbiota in the lower intestine, specifically, for the bacterial population in the ceca of chickens [116] which provide up to 10% of the energy to the bird. In addition, cecal reverse peristalsis produces translocation of the cecal microbiota affecting energy metabolism and performance [133]. The fermentation metabolites produced by the intestinal bacteria depends on the availability of the substrate, fermentation mechanisms and bacteria specie involved in the process [117].

Dietary fiber has a direct, positive effect on the immune response in numerous species by increasing the abundance of some immune cells, specifically T cells, in the gut-associated lymphoid tissue [134], changing the cytokine secretion profile [135, 136] increasing mucosal immunoglobulins and by acting as a prebiotic substrate for beneficial bacteria [137]. For feed ingredients to be considered prebiotics,

they have to meet the following criteria: resistance to an acidic environment (indigestible), fermentation by intestinal microbiota and selective stimulation of beneficial bacterial populations [138]. Based on this concept, dietary fiber is classified as a prebiotic.

A number of studies have found that fiber-rich prebiotics can enhance immune function including direct production of SCFA [139, 140], augmentation of gut burrier function [141], influence on immune mediated inflammatory responses and restoration of the physiological function of bacterial populations.

In human nutrition, multiple benefits have been attributed to dietary fiber, including maintaining normal bowel structure and function, increasing water retention, blood flow, fluid, and electrolyte uptake in colonic intestinal mucosa [128, 142]. Moreover, fiber intake can reduce the risk of metabolic diseases such as obesity, coronary artery disease, diabetes, constipation, inflammatory bowel disease, colitis and colon cancer [128]. In diets rich in protein, the inclusion of dietary fiber such as arabinoxylan-oligosaccharides (AXOS) can potentially decrease the generation of toxic metabolites originated from proteolytic activity and increase the amount of health-promoting bacterial populations [143].

The addition of dietary fiber has also been widely adopted in swine nutrition in order to maximize the nutrient supply and intestinal health [144, 145]. Dietary fiber can change the physiological features of the digesta, most notably modifying the transit time, and the composition of digesta in terms of solubility, fermentability and water retention. Such changes have a direct impact on intestinal functions, bacteria population and fermentation. The inclusion of high to moderate levels of dietary fiber in pigs, remodel the gut microbiota since certain healthy bacteria species such as Lactobacilli and Bifidobacterium tend to increase. The proliferation of lactic acid producing bacteria decrease the pH of the intestinal lumen, resulting in decreased abundance of other pH sensitive enteropathogenic bacteria like *Escherichia coli*, Salmonella, Shigella and Clostridia [144, 145]. Other effects of dietary fiber have been demonstrated. Changes in the gut morphology, most remarkably inducing increases in crypt depth and altering cell division in growing pigs. This effect has been attributed to the trophic nature of SCFA, specifically butyrate [145]. In contrast, fiber is a feed ingredient poorly utilized in poultry nutrition due to antinutritional effects observed from soluble fiber sources that are mainly associated with increased viscosity of digesta and subsequent impair of nutrient absorption and performance parameters [129]. The effects of fiber are variable and depends on the fiber source, particle size, level of inclusion and chemical composition [146]. A number of studies have found that low levels of insoluble fiber can provide benefits from the point of view of gut health by improving nutrient digestibility [147], gizzard functionality, and resulting in modulation of digesta passage and higher nutrient retention [148, 149]. In the literature, a wide range of other effects of dietary fiber have been demonstrated in laying breeders and broilers chickens. In commercial layers supplemented with high fiber ingredients in the diets, environmental improvements have been demonstrated by reducing ammonia concentrations in manure [150], feather pecking [151], cannibalistic behavior and associated mortality [152].

A number of oligosaccharides including lactulose, inulin, galacto-oligosaccharide and mannan oligosaccharides have been proposed to use as prebiotics in chickens. Those non-digestible carbohydrates are metabolized by fermenting bacteria to produce SCFAs. SCFA are nutritional substrates required for an adequate function of the immune system [139]. When xylo-oligosaccharides were supplemented into a broiler chicken diet, the abundance of butyrate-producing bacteria in the colon and ceca, such as Bifidobacterium and Lactobacillus, significantly increased [153].

Similarly, Zhao et al. reported an increase in Lactobacillus counts in excreta when birds were fed with 0.15% inclusion of lactulose [154].

Production of butyrate is considered advantageous to maintain gut health. Butyrate is an important energy source for the enterocytes [140] and is characterized for having immunomodulatory properties. Butyrate can have an anti-inflammatory effect by modulating key inflammatory mediators including the reduction of IFN-γ and NF-kB and the increase in the number of T reg cells and the expression of IL-10 which suppresses the activity of the immune system [155]. Likewise, inulin supplementation in broiler chickens (0.25-0.5%) induces an anti-inflammatory response by decreasing the gene expression of proinflammatory cytokines such as NF-kB, LITAF, IL-6, iNOS and enhances the protective barrier function represented by increased expression of epithelial tightness components including MUC2 and claudin-1 [156].

Other major effects have been shown with the supplementation of oligosaccharides, such as the improvement of growth performance [153], the influence on the intestinal morphology reflected in an increase in crypt depth, villus height and villus area [157] and the reduction of pathogenic bacterial colonization. The increase in pathogen resistance due to prebiotic supplementation is associated with the simultaneous elevation of lactic acid producing bacteria and the decrease in the pH of the intestinal lumen, creating an unfavorable environment for pathogenic bacteria and thereby decreasing the colonization. In fact, a meta-analysis study showed a reduction of 0.61 log10 cfu/g cecal Salmonella spp. in birds treated with lactose and its associated prebiotic products (lactulose, lacto-sucrose, whey and dried milk) [158].

### 5.2 Fatty acids and immune response

Short-chain and medium chain fatty acids play an important role on maintaining intestinal gut health and controlling enteric pathogens [159]. Endogenous metabolic pathways, including beta oxidation of fats, leads to the production of short chain fatty acids (SCFA) such as acetate, propionate and butyrate [160]. Long chain fatty acids can be converted into acetate via acetyl-coA or in propionate via propionyl-CoA [160]. SCFA can modulate multiple cellular metabolic activities by the interaction of nuclear cellular (G-protein couple receptors: GRPs), enzymatic receptors (histone deacetylases: HDACs), serving as a substrate for energy for enterocyte and Krebs's cycle and inducing apoptosis of cells [156]. Through these mechanisms, SCFA modulates gene transcription of cells involved in metabolic pathways, inflammation and immune response. In intestinal cells, butyrate and propionate has the ability to inhibit the HDAC activity which decrease the activation of NFkB transcription factor and subsequently modulating the expression of inflammatory genes [161]. The anti-inflammatory effect of butyrate is produced by preventing the secretion of pro-inflammatory cytokines by macrophages through the NFkb pathway [156].

Regarding the adaptive immune response, butyrate plays an important role in modifying various lymphocyte function including the inhibition of T-cell proliferation, and reduction of the secretion of pro-inflammatory cytokines such as IL-2, IFN-γ and promoting the production of the main anti-inflammatory cytokine, IL-10 [156, 161].

Due to its anti-inflammatory properties, SCFA has been used as a therapeutic alternative for intestinal diseases [162]. Direct delivery of SCFA by encapsulation allows the supplementation without the need for fermentation, increasing the release in the distal gastrointestinal section [163]. Multiple studies have shown that SCFAs are beneficial as a drinking water supplement and feed additive for the control of Salmonella, Campylobacter and Clostridium [164, 165].

In young chickens, *Salmonella enterica spp. enteritidis* cecal colonization significantly decreased when butyric acid was added to the feed [166, 167]. The

addition of SCFA in the drinking water has also been used as an efficient strategy for decreasing the recovery of *Salmonella enterica spp. typhimurium* in the crop and in pre-chilled carcasses at the processing plant [168]. The reduction in colonization of Salmonella by SCFA is related to the regulation of invasion genes (hilA, invF and sipC) located on the pathogenicity island (Sp1-1) [169]. In addition to the antimicrobial activity, SCFA can contribute to disease resistance by enhancing the expression of host defense peptides including Avian β-defensin 9 (*AvBD9*), *cathelicidin B1*, *AvBD3*, *AvBD4*, *AvBD8*, *AvBD10* and *AvBD14* which consequently reduce bacterial growth [170]. However, the ability of SCFA to control Salmonella is highly correlated with acid type and concentration. For example, the feed supplementation of butyric acid in the coated form is more effective in decreasing *Salmonella enterica spp. enteritidis* counts than when using the powder form [166]. Previous studies have also investigated the formic-propionic acid combination at 0.5 and 0.68% respectively, with a significant reduction of *Salmonella enterica spp. kedougou* [171]. Furthermore, the use of a combination of propionic and formic acid decreased the recovery of *Salmonella enterica spp. typhimurium* in the ceca by 3.61 log at 21 days [172]. Similarly, the combination of 1.5% of formic acid and 0.1% of sorbic acid were protective against Campylobacter jejuni colonization during infection trials in broiler chickens by reducing C. jejuni counts in the crop [173].

Among different classes of fatty acids, medium chain fatty acids (MCFA) have reported to be more inhibitory against Salmonella than short chain fatty acids [163]. MCFA are fatty acids composed by 6 to 12 carbons and include caproic, caprylic, capric and lauric fatty acids [174]. The greater antibacterial effect of MCFA is corelated with metabolic differences. Because of its smaller molecular size, MCFA can be absorbed more efficiently and therefore can be utilized more efficiently in the intestinal tract [175]. Indeed, the in-vitro antimicrobial activity of MCFA against Salmonella is observed at very low concentrations (between 10 nM- 50 nM) [176, 177]. Furthermore, in-vivo studies have shown reduction in Salmonella cecal counts with supplementation of caprylic acid [178, 179]. The supplementation with either 0.7 or 1% of caprylic acid significantly reduced the *Salmonella enterica spp. enteritidis* counts in cecal samples of birds fed caprylic acid 7 to 10 days post-challenge in 18 day-old chickens [179]. Another study, showed a reduction in cecal Salmonella *Salmonella enterica spp. enteritidis* counts in ceca, spleen and liver [178] in 3 and 6-week-old chickens. Similarly, the supplementation of caproic acid in broilers decrease the colonization of Salmonella through hilA gen suppression [177].

MCFA acid have also been used for controlling Campylobacter jejuni. Although, studies have shown inconsistent results, caprylic acid at 0.7 and 1.4% has shown to be effective in reducing C. jejuni counts by 3 to 5 log in infected chickens [180].

In conclusion, the application of fatty acids to reduce inflammation and intestinal pathogens is an alternative strategy for poultry nutritionists. Multiple studies support the important role of fatty acids as a modulation of intestinal health. Long chain fatty acids can modulate innate and adaptive immune responses and reduce inflammation produced by systemic diseases. On the other hand, SCFA and MCFA modulate the immune cell function to facilitate the elimination of pathogenic bacteria. Understanding the role of fatty acids in health and disease will increase the effectiveness of these compounds in a wide range of intestinal, metabolic and inflammatory diseases.

## 6. Conclusions

In summary, secretory defense host response and their players including host defense peptides, sIgA and pIgR among others, constitute the first line of intestinal

immune defense and bridge innate and adaptive immune responses at mucosal surfaces. Understanding the complex function and regulation of these immune components may offer new insights into the nutritional prevention and treatment of infectious and inflammatory diseases that originate at mucosal surfaces. Some studies have been addressed the role of key nutrients modulating this secretory defense system and aiding to the host to counteract the noxious effect of harmful microorganism. Based on that, nutrition would be considered as an important strategy in the reduction of antibiotic growth promotants. However, more studies are needed to understand the effects of nutrients on gut immune response against pathogens.

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