Regulatory Effects of Nutritional Factors on Intestinal Mucosal Immunity in Non-Ruminant Animals (Postprint)

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Abstract

The intestine possesses the function of isolating luminal contents from the internal environment of the organism, preventing pathogenic antigens (intraluminal bacteria, toxic substances, food antigens, etc.) from invading the submucosal tissues, thereby maintaining relative stability of the internal environment. In this process, the mucosal defense system plays a crucial role. This article primarily reviews the composition, structure, classification, and main immune factors of the intestinal mucosal immune system, as well as the regulatory effects of nutritional factors on intestinal mucosal immunity, aiming to provide a reference for the effects of nutrients and non-nutrients on intestinal immune function and the mechanisms of immunomodulation in non-ruminant animals.

Full Text

Regulation of Nutritional Factors on Intestinal Mucosal Immunity in Non-Ruminant Animals

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Abstract: The intestine serves as a critical barrier that isolates luminal contents from the internal environment, preventing pathogenic antigens (intestinal bacteria, toxic substances, food antigens, etc.) from invading submucosal tissues and thereby maintaining internal homeostasis. The mucosal defense system plays a vital role in this process. This review summarizes the composition and structure of the intestinal mucosal immune system, its classification, major immune factors, and the immunomodulatory effects of nutritional factors on intestinal mucosal immunity, aiming to provide a reference for understanding the

mechanisms by which nutrients and non-nutrients regulate intestinal immune function in non-ruminant animals.

Keywords: non-ruminant animals; intestinal mucosa; immune factors; immune mechanisms; nutritional factors

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The mucosa, as the first line of defense in the immune system, directly contacts the external environment and undertakes complex immune tasks. It must tolerate beneficial commensal microorganisms and food antigens while mounting immune responses against pathogenic microbial invasion. With advances in biotechnology and molecular biology, mucosal immunity has regained attention and developed rapidly. Mucosal immunity refers to the immune responses occurring at mucosal surfaces of body cavities that communicate with the external environment, primarily comprising mucosa-associated lymphoid tissues of the gastrointestinal tract, respiratory tract, urogenital tract, and certain exocrine glands (such as salivary and lacrimal glands). These mucosal barriers represent the largest interface between the body and the external environment and serve as important entry points for pathogens, making mucosal immunity extremely important in host defense.

The mucosal immune system recognizes pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) to generate immune responses that clear pathogens and maintain homeostasis. Lymphocyte migration and cytokine imbalance in the intestinal mucosa are implicated in the pathogenesis of inflammatory bowel diseases, including intestinal allergies and Crohn' s disease. Since dietary components are closely related to intestinal mucosal immune function, dietary control is important for treating inflammatory bowel disease. Research indicates that anti-nutritional factors (ANF) in peas increase the number of T cells in the jejunal epithelium of broilers, triggering mucosal immune responses in the jejunum. Legumes containing ANFs indirectly affect the intestinal immune system by altering intestinal microbial community composition through increased fermentation of oligosaccharides or non-starch polysaccharides.

1. Composition and Structure of the Intestinal Mucosal Immune System

Intestinal mucosal immunity constitutes the first line of defense against infection. When the intestinal mucosa is compromised by external factors, its immune function weakens, allowing pathogens such as bacteria and viruses to invade easily, leading to indigestion, diarrhea, and even life-threatening conditions. The intestine is the primary site for food digestion and nutrient absorption, while the intestinal mucosa also serves as a major portal for pathogen entry. Mucosal

immunity represents a protective mechanism formed through long-term evolutionary interactions between the host and pathogens.

The intestinal mucosal immune system is a highly specialized system. Based on functional and distributional characteristics, mucosal immune cells can be divided into two components: gut-associated lymphoid tissue (GALT), which serves as the activation-inducing site for immune cells, and diffuse immune cells, which function as effector sites where immune responses occur. GALT primarily includes microfold cells (M cells) and Peyer' s patches (PP), while diffuse immune cells mainly comprise intestinal epithelial cells (IEC), intraepithelial lymphocytes (IEL), and lamina propria lymphocytes (LPL).

2. Classification of Intestinal Mucosal Immunity

The intestinal mucosal immune barrier is one of the most important barriers in animals and humans. Intestinal mucosal immunity primarily generates local immune responses upon antigen stimulation to neutralize antigenic substances and prevent damage to the host. Functionally, the intestinal mucosal barrier represents the first natural line of defense against pathogen invasion through the cooperative action of the mucus layer, intestinal cells, and tight junctions. Intestinal mucosal immunity is divided into innate immunity and adaptive immunity. Innate immunity is primarily executed by innate immune cells, while adaptive immunity mainly occurs in gut-associated lymphoid tissues such as Peyer' s patches, which constitute tertiary lymphoid organs.

2.1.1 Intestinal Mucosal Epithelial Cells

In addition to forming a natural mechanical barrier, intestinal mucosal epithelial cells generate innate immune responses through PRRs that recognize pathogens and their toxins, thereby enhancing the killing and clearance of pathogenic microorganisms. Toll-like receptors (TLRs) and NOD-like receptors (NLRs) play important roles in mucosal epithelial cell immune responses.

2.1.2 Mononuclear Phagocytes and Dendritic Cells (DC)

Mononuclear phagocytes and DC migrate to the intestinal mucosa through blood circulation and secrete large amounts of the anti-inflammatory cytokine interleukin (IL)-10, which inhibits immune responses and maintains immune homeostasis. DCs in the intestinal mucosa are specifically characterized by expression of integrin CD103. CD103 DCs continuously capture antigens transported by goblet cells, secrete IL-10 and transforming growth factor- (TGF-), and migrate to mesenteric lymph nodes (MLN) to activate naïve T cells, converting them into regulatory T cells (Treg).

2.1.3 Innate Lymphoid Cells (ILC)

ILCs are derived from lymphoid progenitor cells and differ from conventional lymphocytes in that they lack antigen receptors generated by gene recombination. ILCs can be divided into three subsets: 1) ILC-I expresses transcription factor T-bet and primarily secretes interferon- (IFN-) and tumor necrosis factor- (TNF-); 2) ILC-II expresses GATA-binding protein 3 (GATA3) and primarily secretes IL-5 and IL-13; and 3) ILC-III expresses retinoid-related orphan receptor t (ROR t) and primarily secretes IL-17 and IL-22.

2.2 Intestinal Mucosal Adaptive Immunity

Mucosal adaptive immunity primarily occurs in mucosa-associated lymphoid tissue (MALT), including Peyer' s patches and tonsils. Peyer' s patches represent tertiary lymphoid tissue in the intestinal mucosa and constitute a functional site capable of executing complete immune responses, harboring CD4 T cells, CD8 T cells, B cells, DCs, and macrophages that execute adaptive immunity. Intestinal antigens are primarily transported to MALT through M cells to activate B cells and trigger humoral immune responses, while also being captured by DCs to activate corresponding T cells. Activated T cells can rapidly migrate to MLN to induce stronger immune responses. Numerous CD4 T cells in the lamina propria secrete cytokines that downregulate immune reactions, while sensitized B cells that have migrated to the lamina propria undergo isotype switching to immunoglobulin A (IgA). IgA is released to the serosal cell exterior, binds to the secretory component produced by mucosal epithelial cells, and forms secreted immunoglobulin A (sIgA) complexes that are ultimately secreted onto mucosal or serosal surfaces to exert immune effects. Crosstalk between intestinal epithelial cells and adaptive immune cells may play an important role in intestinal mucosal immunity.

3. Major Factors in Intestinal Mucosal Immunity

3.1 sIgA

sIgA dominates the normal function of intestinal mucosal immunity. As the first immune barrier, mucosal immunity holds a special position in the immune system. The intestinal mucosal barrier prevents harmful substances in the intestinal lumen from crossing into other tissues, organs, and blood circulation, with humoral immunity centered on sIgA being a crucial component. sIgA is the primary immunoglobulin of the intestinal mucosal immune barrier, possessing biological functions such as strengthening the intestinal immune barrier and preventing pathogen invasion, serving as an important defense front against bacterial adhesion and colonization in the intestinal mucosa. sIgA is composed of two or more IgA monomers, typically consisting of two IgA monomers, one J chain, and one secretory component (SC).

The prerequisite for microbial infection is adhesion to mucosal tissue; therefore,

inhibiting microbial adhesion is one of the most important protective functions of mucosal immunity. sIgA prevents pathogenic microorganisms from adhering to mucosal epithelial cell surfaces through several mechanisms: 1) sIgA agglutinates pathogenic microorganisms, causing them to lose motility and adhesion capacity; 2) sIgA blocks specific binding sites on microbial surfaces after binding, preventing adhesion; and 3) sIgA forms immune complexes with pathogenic microbial antigens, stimulating goblet cells in the digestive tract mucosa to secrete large amounts of mucus that "wash" the mucosal epithelium, thereby preventing microbial adhesion. sIgA effectively neutralizes harmful substances such as bacteria, viruses, toxins, and enzymes within the mucosal epithelium, captures pathogens in the mucosal inner layer, and forms immune complexes for excretion. The local presence of specific sIgA in mucosa reduces absorption of corresponding antigens after exposure to soluble antigens from the external environment without affecting unrelated antigens. sIgA enhances the antibacterial effects of lactoperoxidase and the lactoperoxidase system against several mucosal pathogens, enhances antibody-dependent cellular functions through mucosal lymphoid tissue, arms luminal lymphocytes to improve direct bactericidal capacity, and synergizes with antimicrobial substances such as lactoferrin and lysozyme in secretions. sIgA antibodies can bind to M cells and be transported to mucosal lymphoid tissue, a process that enables sIgA or sIgA-antigen complexes to interact with lymphocytes or antigen-presenting cells, exposing sIgA binding sites.

3.2 Cytokines

The intestinal mucosal immune barrier produces various cytokines, including lymphokines, chemokines, growth factors (GF), TNF, IL, and IFN. The diverse regulatory functions of cytokines are closely related to their concentrations and microenvironment. Recent research has focused on cytokines such as IL-6, IL-10, IL-12, IL-17, IFN- , and TNF- .

Immune responses in intestinal mucosal sites are dominated by T helper (Th) 2 cells. CD4 Th2 cells residing in the lamina propria secrete multiple Th2 cytokines, including TGF- , IL-4, IL-5, IL-6, and IL-10. After capturing pathogens, DCs secrete TGF- , which is pleiotropic—not only inducing naïve CD4 T cells to become iTreg and suppressing immune responses but also, in combination with IL-6, promoting differentiation of naïve CD4 T cells into Th17 cells that secrete IL-17A and IL-22, generating inflammatory responses. IL-4 activates resting B cells and plays a key role in inducing both local and systemic antibody responses, while IL-5 and IL-6 exert special functions primarily at mucosal sites. IL-6 plays an important role in promoting IgA responses in the intestine and respiratory tract. IL-10 is a cytotoxic T lymphocyte (CTL) differentiation factor and B cell activation factor that inhibits macrophage secretion of TNF, IL-1, IL-6, and chemokines, suppresses macrophage assistance to T cells, reduces T cell proliferation capacity and CTL killing activity, inhibits cell-mediated immune responses, and relatively enhances humoral immune re-

sponses. IL-4, IL-5, and IL-6 synergistically induce B cell differentiation into IgA-secreting plasma cells that enter mucosal surfaces through secretory component mediation to neutralize antigenic substances and clear foreign antigens to protect the host. IL-12 is a heterodimeric complex composed of (P35) and (P40) subunits that activates natural killer (NK) cells to produce IFN- . As an important pro-inflammatory cytokine, IL-12 is a key regulatory molecule linking innate and adaptive immunity, primarily acting on target cell receptors through the Janus kinase (Jak)/signal transducer and activator of transcription (STAT) signaling pathway. IL-17 exerts biological effects by binding to IL-17 receptors on target cell membranes. Since IL-17 receptors are widely expressed in various cells throughout the body, IL-17 acts on multiple cell types with pleiotropic functions. IL-17 induces fibroblasts and epithelial cells to secrete pro-inflammatory cytokines and chemokines, recruits neutrophil infiltration to resist pathogen infection, and also participates in the development of various autoimmune diseases. IFN- is produced by activated T cells and NK cells and promotes CD4 cell activation by stimulating antigen-presenting cells to express major histocompatibility complex class II (MHC II) molecules. TNF- is a pro-inflammatory factor primarily produced by activated macrophages that exerts biological effects by binding to TNF-R1 or TNF-R2 receptors, causing apoptosis and cytokine release.

4. Regulation of Nutritional Factors on Intestinal Mucosal Immunity

The intestinal mucosal immune barrier is influenced by various nutritional factors, and its maintenance requires nourishment from luminal nutrients. Certain substrates such as glutamine (Gln), arginine, short-chain fatty acids (SCFAs), and nucleotides play important regulatory roles in intestinal mucosal immunity.

4.1 Glutamine

Gln is one of the most abundant amino acids in animals and is conditionally essential, serving as an important indicator of immune function. Gln can alleviate intestinal mucosal barrier damage in severe acute pancreatitis and inhibit inflammatory response activation. As an important energy donor for intestinal mucosal epithelial cells and lymphocytes, Gln is a major energy substrate for intestinal epithelial cells. Gln also serves as a precursor for purine and pyrimidine synthesis, playing an important role in immune cell proliferation. During severe stress from trauma, infection, or fatigue, Gln in intestinal mucosal epithelial cells is rapidly depleted. When the intestine lacks stimulation from food or digestive juices, or when Gln is deficient, intestinal mucosal atrophy occurs with sparse, shortened villi or even sloughing, shallow crypts, increased intestinal mucosal permeability, and compromised intestinal immune function. Gln can improve animal production performance while maintaining intestinal mucosal barrier function. Studies have shown that Gln supplementation effectively protects intestinal mucosal barrier function in advanced gastric cancer patients undergoing

perioperative chemotherapy, with significant clinical value in improving matrix metalloproteinase-2 (MMP-2) and MMP-9 activity, enhancing immune function, and reducing morbidity. Alanine (Ala)-Gln supplementation increases IL-4 and TGF- content as well as mRNA expression of poly-immunoglobulin receptors (PIGR) and J chain in intestinal tissues of plateau-trained rats, promoting sIgA synthesis and secretion and mitigating intestinal humoral immune function damage caused by plateau training. Dong et al. found that dietary supplementation with 0.8% Gln significantly improved duodenum and oviduct development and increased egg production rate in laying hens. Gln provides energy for rapidly dividing cells such as intestinal mucosal epithelial cells and activated lymphocytes, playing an irreplaceable role in promoting repair of damaged intestines and maintaining normal local immune function. When lymphocytes isolated from broiler jejunum were cultured with Gln for 24 hours, results showed that Gln significantly improved lymphocyte proliferation. When Gln concentration exceeded 50 g/mL, its inhibitory effect on broiler intestinal lymphocyte proliferation was most significant, helping maintain immune system balance while significantly improving malondialdehyde (MDA) content and catalase (CAT) activity, benefiting antioxidant capacity in animals.

4.2 Arginine

The physiologically active form of arginine in animals is L-arginine, which participates in the synthesis of various bioactive substances such as creatine, polyamines, nitric oxide (NO), Gln, and pyrimidines, and affects the release of multiple endocrine hormones. Arginine and its metabolites (such as NO and polyamines) play important roles in immune defense and regulation. Arginine can improve local blood perfusion by increasing NO synthesis in intestinal tissue, reduce lipid peroxidation damage to the intestinal mucosa, effectively stimulate proliferation of immune cells in Peyer' s patches within the lamina propria, and promote intestinal IgA secretion. Arginine is an essential amino acid for piglets. Research shows that arginine increases the number of IgA-secreting cells, CD8 , and CD4 T cells in the ileum of weaned piglets, prevents lipopolysaccharide (LPS)-induced increases in mast cell numbers, protects and enhances intestinal mucosal immune barrier function, and maintains intestinal integrity in weaned piglets. Arginine and vitamin E synergistically increase cellular and humoral immune function in broilers, improving disease resistance. Gao et al. demonstrated that feeding arginine solution significantly increased inducible nitric oxide synthase (iNOS) activity and IL-2, IL-4, and sIgA content in broiler jejunum, as well as mRNA expression of TLR-2, TLR-4, and iNOS and iNOS protein abundance in intestinal mucosa. In ovo injection of L-arginine improved post-hatch small intestine development and barrier function in broilers, possibly through activation of the mammalian target of rapamycin (mTOR) pathway.

4.3 Short-Chain Fatty Acids

SCFAs are organic acids containing 2-5 carbon atoms, primarily produced by bacterial fermentation of oligosaccharides, polysaccharides, peptides, proteins, and glycoproteins in the intestine. As major metabolites of intestinal flora, SCFAs regulate intestinal epithelial cells and various immune cells within intestinal mucosal tissue, participating in both innate and adaptive immune responses, alleviating inflammatory reactions, inhibiting tumor cell proliferation, and maintaining intestinal mucosal homeostasis. SCFAs participate in intestinal disease development by affecting intestinal epithelial cell and immune cell functions, recognizing TLRs, activating G protein-coupled receptors (GPCRs), and inhibiting histone deacetylase (HDAC) activity. Butyrate, a component of SCFAs produced by bacterial fermentation in the large intestine, is the preferred oxidizable fuel for intestinal tissue cells. Studies show that acetate and propionate significantly reduce mucosal paracellular permeability and net transepithelial fluid flux while increasing mucosal bicarbonate secretion. n-3 and n-6 polyunsaturated fatty acids (PUFAs) reduce -proteobacteria overgrowth while promoting Bacteroides growth; n-3 PUFAs are superior to n-6 PUFAs in improving ileal tissue lysozyme activity after hemorrhagic shock resuscitation (HSR) in mice. PUFAs, particularly n-3 PUFAs, can partially improve innate immunity in mouse intestinal mucosa after HSR. Acetate inhibits caspase-3 activity and BAX expression, promoting cell survival, possibly by upregulating the key gastric defense factor mucin (MUC)5AC. Acetic acid protects the stomach in ethanol-induced gastric injury through synergistic multi-pathway effects, including inhibiting gastric oxidation, inflammation, and apoptosis while promoting MUC5AC expression. Increased numbers of intestinal regulatory T cells and luminal IgA production correlate with retinaldehyde dehydrogenase 1 (RALDH1) expression in small intestinal epithelial cells and vitamin A conversion enzyme activity in mesenteric lymph node DCs. Dietary fiber consumption alters the structure of SCFA-producing microorganisms and SCFA composition in the small intestine. Although SCFAs benefit intestinal mucosal immunity, their specific roles in intestinal flora composition and function and their relationship with intestinal flora-associated diseases require further investigation.

4.4 Nucleotides

Nucleotides are compounds composed of purine or pyrimidine bases, ribose or deoxyribose, and phosphate, with multiple important biological functions including genetic material synthesis, cell signal transduction, energy metabolism participation, and coenzyme activity. Nucleotides participate in energy transfer and are precursors for nucleic acid synthesis, involved in various physiological regulation processes with growth-promoting and intestinal development-improving effects, making them semi-essential nutrients. Dietary nucleotides promote normal intestinal development in healthy animals through nucleotide carrier regulation, modulating the balance between cell proliferation and apoptosis and maintaining normal immune function. Compared with salvage synthe-

sis, de novo nucleic acid synthesis consumes substantial energy and substrates. During rapid growth, disease, trauma, or stress, dietary nucleotide supplementation promotes immune system development, maintains intestinal health, and conserves energy while protecting the intestine and promoting repair after damage through nucleotide carrier regulation. Studies show that nucleotide supplementation in pig diets significantly reduces feed conversion ratio, increases duodenal villus height, jejunal lactase and maltase activities, peripheral blood leukocyte count, serum IgA and IL-1 content, and mRNA expression of ileal TLR-9, TLR-4, and toll-interacting protein (Tollip), while significantly increasing mRNA expression of ileal tight junction proteins (Claudin-1 and ZO-1). CpG oligodeoxynucleotides (CpG ODN) significantly increase IL-12, TNF- , and TLR-9 mRNA expression and enhance nuclear factor- B (NF- B) signaling activation in LPS-stimulated cells. Additionally, nucleotides are widely used as immune enhancers in aquaculture. Adding nucleotides to shrimp feed significantly reduces serum superoxide dismutase (SOD), iNOS, and lysozyme activities in shrimp. However, the mechanisms of nucleotide action on animal intestines (enterocytes, genes, etc.), exogenous nucleotide requirements at different developmental stages, maximum tolerance levels, and dose-response relationships require in-depth research.

4.5 Probiotics

The intestinal microecosystem participates in the development of the intestinal mucosal immune system, promotes sIgA synthesis and secretion, and interacts with intestinal mucosal immune cells to maintain intestinal homeostasis, playing important roles in the development of inflammatory bowel disease, irritable bowel syndrome, and allergic diseases. Intestinal microorganisms are closely related to human health, with 70%-80% of immune cells distributed in GALT. Immunosuppression can promote engraftment of transplanted microbiota, indicating that intestinal microbial communities influence the intestinal mucosal immune system. Oral administration of *Lactobacillus plantarum* NCU116 isolated from kimchi to cyclophosphamide-treated mice improved intestinal villus height and crypt depth, mucin expression, goblet cell numbers, and colonic microbiota diversity while increasing intestinal SCFA levels and reducing ammonia content in colonic feces. *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and other microorganisms can regulate the intestinal environment, inhibit or kill gastrointestinal pathogens, improve intestinal microecological balance, modulate intestinal mucosal immunity, and maintain intestinal barrier function. Related mechanisms include producing bactericidal substances against gastrointestinal pathogens and harmful microorganisms, competing with pathogenic microorganisms for binding sites on intestinal epithelial cells and mucins, and activating the immune system. *Lactobacillus reuteri* biofilm-secreted factors can inhibit human TNF production by LPS-activated monocytes, while *L. reuteri* biofilms secrete reuterin (antimicrobial glycerol derivatives). Oral administration of *Lactobacillus casei* CRL431 to mice primarily activates innate immune-related intestinal immune system components (such as macrophages and DCs) with less T cell in-

volvement through increased cell markers CD-206 or TLR-2 receptor expression, stabilizing intestinal homeostasis. This suggests that lactobacilli can improve immune responses without triggering specific T cell-dependent IgA responses. In another study, *Lactobacillus paracasei* enhanced interactions between CD4 regulatory T cells and DCs in Peyer' s patches, promoted CD4 T cell and B cell proliferation, and increased mRNA expression of IL-1 , IL-10, IL-12, IFN- , and TNF- . Probiotic immunomodulatory components comprise multiple effector molecules including surface layer proteins, cell wall polysaccharides, adhesins, teichoic acids, and heat shock proteins, while other components remain overlooked or yet to be identified. Although in vitro studies have identified important candidate effector molecules [such as elongation factor Tu (EF-Tu)], the mechanisms of probiotic intervention on mucosal immunity in vivo still need determination. Baseline "healthy"conditions may vary according to age, sex, breed, diet, and multiple environmental factors. While some parameters (leukocytes, cytokines) are understood, baseline parameters including quantitative "normal" ranges for immune biomarkers remain to be established.

4.6 Vitamins

Vitamin A (VA) is an important micronutrient that, besides maintaining normal vision, promotes growth and development and epithelial tissue proliferation and differentiation. VA serves as an important immunomodulator that plays significant roles in infectious diseases, with deficiency reducing both cellular and humoral immune functions. Reports indicate that VA deficiency significantly reduces fish growth performance, increases enteritis incidence, decreases intestinal innate humoral immune responses, and exacerbates intestinal inflammation. Different immune responses in proximal, middle, and distal intestine are partially mediated by canonical NF- B signaling and p38 mitogen-activated protein kinase (MAPK) pathways. VA can also upregulate the Nrf2/Keap1 signaling pathway in fish intestine, body, and gills, increasing gene expression and activity of antioxidant enzymes such as Cu-ZnSOD, enhancing non-enzymatic antioxidants like glutathione (GSH) and VA content, improving free radical scavenging capacity, reducing reactive oxygen species (ROS) content, and decreasing oxidative damage. VA may also downregulate caspase-8 and caspase-9 mRNA expression through the TOR signaling pathway, thereby downregulating caspase-3 (but not caspase-7) mRNA expression to inhibit apoptosis in intestinal, body, and gill cells, maintaining cellular structural integrity.

Vitamin D (VD) is a hormone synthesized primarily in human skin upon ultraviolet radiation stimulation. VD' s role in regulating calcium and phosphorus balance is well established, as it balances calcium and phosphorus absorption and storage and prevents rickets. Beyond its endocrine role in bone metabolism, VD has significant immunomodulatory effects, including enhancing monocyte/macrophage microbicidal capacity and downregulating inflammatory cytokine production by T lymphocytes. VD maintains intestinal mucosal barrier integrity by enhancing intercellular junctions that control mucosal per-

meability and reduce pro-inflammatory cytokines such as IL-8. Additionally, vitamin D receptor-mediated signal transduction inhibits inflammation-induced intestinal epithelial cell apoptosis, and maintaining adequate VD levels is essential for healthy gut microbiota development. VD inhibits necrotizing enterocolitis (NEC) development in rats by upregulating intestinal epithelial tight junction protein expression. Studies show that 1,25-dihydroxyvitamin D regulates intestinal microbiota and stromal fibroblasts while possessing anti-tumor, immune-regulating, and anti-obesity functions.

Niacin (vitamin B , nicotinic acid) dilates peripheral blood vessels and maintains normal skin and digestive organ functions, primarily participating in carbohydrate, lipid, and protein metabolism through nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), playing important roles in functional metabolic reactions. Niacin participates in over 200 dehydrogenase reactions in fish as a coenzyme, essential for normal digestive organ function. Research indicates that niacin deficiency reduces intestinal lysozyme and acid phosphatase (ACP) activities and complement 3 (C3) content in grass carp, while downregulating hepatic mRNA expression of LEAP-2, hepatocyte growth factor, IL-10, TGF- 1, I B inhibitor, TNF- , IL-1 , IFN- 2, IL-8, NF- B P65, I B kinase (IKK), IKK , and IKK .

4.7 Minerals

Appropriate mineral levels protect the body, while excessive amounts are toxic. Reducing mineral supplementation appropriately can improve intestinal absorption rates and release minerals accumulated in the liver. However, trace element deficiency reduces animal performance and immune responses. Selenium (Se) participates in glutathione peroxidase (GSH-Px) composition and antioxidant functions, strongly reducing hydrogen or lipid peroxides to protect cell membrane structure and function, with cardiovascular protective, immune-enhancing, and heavy metal toxicity-reducing effects. Zhang et al. reported that different Se and vitamin E levels improved serum and liver antioxidant capacity, significantly reduced peroxide content, improved immune stress status, and enhanced antioxidant capacity while reducing intestinal NO content and iNOS activity, providing positive intestinal protection. Zinc (Zn) is an important nutrient for lipid, carbohydrate, DNA, RNA, and protein synthesis and degradation, with important roles in disease resistance, wound healing, and epithelial integrity maintenance. Studies show that Zn upregulates protein kinase C (PKC) signaling molecules through GPCR39 activation, promoting tight junction protein ZO-1 and cadherin expression. Additionally, Zn promotes cell differentiation and ZO-1 mRNA expression through the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mTOR pathway, enhancing intestinal barrier function and improving Caco-2 monolayer epithelial cell integrity.

4.8 Polysaccharides and Oligosaccharides

Polysaccharides are widely present in animal cell membranes and plant and microbial cell walls, possessing important biological activities such as participating in cytoskeleton formation and serving as components of various endogenous bioactive molecules. As immune promoters and modulators, polysaccharides have antibacterial, anti-tumor, antioxidant, and antiviral activities. Research shows that crude polysaccharides (KPV-0) extracted from Korean persimmon vinegar are non-cytotoxic to intestinal epithelial Caco-2 cells, can be transported across Caco-2 cell monolayers in vitro co-culture systems, significantly increase IgA production by Peyer' s patch cells, and elevate TGF- 1 and IL-6 levels. In vivo oral administration results show that KPV-0 significantly increases IgA content in intestinal fluid and feces. Both unfermented Yupingfeng polysaccharides (UYP) and fermented Yupingfeng polysaccharides (FYP) significantly promote growth and enhance immune activity in weaned rex rabbits, improve intestinal flora balance, and maintain intestinal barrier structure and function integrity. FYP increases intestinal flora diversity and cellulolytic bacterial abundance while reducing *Streptococcus* and *Enterococcus* abundance. In the gastrointestinal tract, particularly the foregut, FYP maintains intestinal barrier integrity and function by upregulating mRNA expression of tight junction proteins, polymeric immunoglobulin receptors, trefoil factors, and epidermal growth factor in the jejunum and ileum. Extracellular polysaccharides (EPS1-1) extracted from *Rhizopus nigricans* fermentation broth enhance immunity in immunosuppressed mice, resist hydrolysis in artificial stomachs, regulate intestinal microbiota, increase SCFA content in feces of colorectal cancer mice, increase colon villus height, villus height/crypt depth ratio, and the number of acidic mucus-secreting goblet cells. *Hericium erinaceus* polysaccharides (HEP) significantly improve intestinal morphology and related indicators in ducklings, inhibit reductions in intestinal mucosal epithelial lymphocytes, goblet cells, and mast cells caused by Muscovy duck reovirus (MDRV) infection, and significantly increase sIgA, IFN- , and IL-4 secretion, enhancing intestinal mucosal immune function.

Oligosaccharides, also called oligosaccharides or oligosaccharides, can be divided into functional and common oligosaccharides based on biological function. Functional oligosaccharides have special biological functions, cannot be absorbed by human or animal intestines, and cannot be utilized by most harmful intestinal bacteria, but can be fermented and utilized by beneficial intestinal bacteria such as *Bifidobacterium* and *Lactobacillus*, thereby promoting their growth and proliferation, representing a class of oligosaccharides beneficial to intestinal health. Dietary supplementation with alginate oligosaccharides (ALGO) significantly increases serum IL-10, immunoglobulin G (IgG), and IgA content, SOD and CAT activities, and total antioxidant capacity (T-AOC) in weaned piglets, increases intestinal *Bifidobacterium* and *Lactobacillus* numbers while reducing total bacteria and *E. coli* numbers, significantly increases small intestinal sIgA content and villus height, enhances disaccharidase (lactase and sucrase) activity, and

upregulates tight junction protein ZO-1 mRNA expression. Dietary mannan oligosaccharide supplementation significantly increases lymphocyte subset proportions (except CD8 T cells) in weaned piglets during the final two weeks of the trial, while significantly reducing CD45 , CD4 , and CD8 T cell proportions throughout the trial period. On day 35 of the trial, total bacterial load in the jejunum decreased while the number of naïve T cells (CD45RA T) in ileal Peyer' s patch interfollicular and follicular regions increased.

5. Summary

Intestinal mucosal immunity is an important defense line against infection and a critical portal for establishing and maintaining homeostatic balance between the host and external environment. With continuous scientific research, implementing targeted and effective measures to improve intestinal mucosal immune defense and strengthen barrier function has become a research focus at the intersection of nutrition and immunology. Currently, further in-depth research is needed on specific nutrients involved in intestinal microbial regulation and specific symbiotic bacteria involved in immune modulation to reveal the underlying mechanisms of interaction between microbial immunity and nutritional regulation in animal intestines, providing a more scientific theoretical basis for the effects and mechanisms of nutrients and non-nutrients on animal intestinal immune function.

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