Gut Health in Aquatic Animals and Feed Additives (Postprint)

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Abstract

Gut health status determines animal health, gut health is one of the critical factors ensuring rapid animal growth, and certain exogenous feed additives can improve animal gut health. This article summarizes additives reported domestically and internationally that can improve animal gut health in livestock, poultry, and aquatic animals, including functional amino acids, zinc, fatty acids, probiotics, and polysaccharides, reviews their effects on intestinal structure and function, and elaborates on partial mechanisms of action, aiming to provide references for improving gut health in aquatic animals.

Full Text

Research Advances in Intestinal Health and Feed Additives for Aquatic Animals

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Abstract: Intestinal health is a critical determinant of overall animal health and represents one of the key factors ensuring rapid growth in animals. Certain exogenous feed additives can effectively improve intestinal health. This review summarizes reported feed additives that enhance intestinal health in livestock, poultry, and aquatic animals, including functional amino acids, zinc, fatty acids, probiotics, and polysaccharides. We examine their effects on intestinal structure and function and elucidate some of the underlying mechanisms, providing a theoretical basis and reference for improving intestinal health in aquatic animals.

Keywords: intestinal health; feed additives; aquatic animals

The intestine serves as the primary site for nutrient digestion and absorption in animals, comprising four structural layers: the mucosa, submucosa, muscularis, and serosa. The mucosal layer, being the outermost component, directly contacts nutrients and intestinal microorganisms, making the intestinal mucosal barrier system essential for preventing invasion by luminal toxins and bacteria. Normal morphological structure and function of the intestinal mucosa are therefore critically important for organismal health. However, numerous factors can cause mucosal damage, including malnutrition, various enteritides, parenteral nutrition, and endotoxin infection. Once normal intestinal function is compromised—through disruption of mucosal integrity, barrier dysfunction, or immune system dysregulation—various pathological conditions may arise, such as reduced appetite, low feed intake, impaired development, and decreased nutrient digestion and absorption capacity. In aquatic animals, intestinal health is influenced by feed composition, aquaculture water environment, and intestinal microbiota. For instance, high dietary soybean meal content can induce oxidative damage and structural destruction in fish intestine due to its antinutritional factors, triggering enteritis. Typical symptoms of enteritis in fish include: (1) thinning of mucosal folds with vacuolation or disappearance of nuclei in absorptive cells; (2) thickening of the lamina propria within mucosal folds, with infiltration of numerous inflammatory cells (macrophages or eosinophils) into both the lamina propria and mucosal epithelium; and (3) increased goblet cells in the mucosal epithelium. Consequently, nutritional intervention through exogenous measures to improve intestinal function is essential for aquatic animal health. This review focuses on the effects of feed additives on intestinal health in aquatic animals to provide references for future applications.

1 Functional Amino Acids

Functional amino acids are defined as amino acids that perform special functions beyond protein synthesis. They are essential for normal growth and physiological maintenance and serve as precursors for various bioactive substances. Nutritionally, they may be either essential or non-essential amino acids, including glutamine, arginine, branched-chain amino acids, tryptophan, proline, glycine, histidine, aspartic acid, asparagine, and sulfur-containing amino acids. Research demonstrates that arginine, glutamine, threonine, tryptophan, and lysine can promote intestinal development and facilitate repair of damaged intestinal mucosa.

1.1 Glutamine

Glutamine, an aliphatic neutral amino acid, is the most abundant amino acid in animals. It serves as a critical energy substrate for rapidly proliferating cells (including epithelial cells, lymphocytes, tumor cells, and fibroblasts) and is an important precursor for protein, nucleotide, and amino sugar synthesis. As the primary energy source for small intestinal mucosa, glutamine content substantially exceeds that of glucose and fatty acids, making it essential for synthesizing polyamines and glutathione that maintain mucosal structure and function. Numerous studies confirm that glutamine utilization by small intestinal mucosa is indispensable for maintaining structural integrity and functional capacity. Under pathological conditions, oral or intravenous glutamine supplementation can promote recovery from intestinal damage caused by multiple organ system failure, endotoxemia, skin burns, weaning, and cancer. Research on rodents and pigs shows consistent beneficial effects; for example, 2% dietary glutamine improves growth performance and intestinal function in weaned piglets, while 3% glutamine supplementation reduces intestinal injury in rats with traumatic brain injury.

Due to glutamine' s instability in aqueous solution and intolerance to high-pressure sterilization, direct use of free glutamine is limited in clinical medicine and practical production. Recently, synthetic glutamine-containing dipeptides such as alanine-glutamine (Ala-Gln) and glycine-glutamine (Gly-Gln) have been widely applied because of their superior aqueous stability, high-pressure sterilization tolerance, and rapid release of glutamine upon entering the body. Studies in weaned piglets fed plant-based diets show that 1.01 g/kg BW Ala-Gln supplementation for 14 days significantly improves immune function, intestinal structure, and growth compared to controls. Similarly, mice with malnutrition-induced enteritis showed markedly improved intestinal function when administered drinking water containing 111 mmol/L Ala-Gln for 21 days. These beneficial effects of glutamine-containing dipeptides on intestinal function have been confirmed in studies using mouse and porcine intestinal epithelial cells.

Glutamine was the first amino acid discovered to activate key kinases in intestinal cell signaling pathways. It enters cells primarily through sodium-dependent amino acid transporters, specifically amino acid/carnitine transporters B , (ATB ,) and neutral amino acid transporter B(0)-like (ASCT2), which maintain high glutamine influx into small intestinal absorptive cells by regulating transporter expression levels. The mechanisms underlying glutamine' s efficacy in diarrheal diseases and enteritis include: (1) glutamine transport coupling with sodium absorption, and (2) complementary sodium absorption effects to those of glucose. ASCT2, a high-affinity neutral amino acid transporter for glutamine, functions as an exchanger and plays a crucial role in glutamine uptake. Additionally, glutamine is considered an important factor mediating the mitogen-activated protein kinases (MAPKs) signaling pathway in porcine intestinal cells, serving as a signal to enhance cell survival in the intestine and other vital organs while inhibiting apoptosis and exerting anti-inflammatory effects.

Research on glutamine' s effects on aquatic animal intestinal health has also been reported. Pohlenz et al. found that dietary glutamine supplementation for 10 weeks improved intestinal morphology and enterocyte migration rates in channel catfish (*Ictalurus punctatus*) without affecting growth or plasma amino acid content. In juvenile Jian carp (*Cyprinus carpio* var. Jian), 1.2% dietary glutamine enhanced growth performance, feed utilization, intestinal weight, villus length, and digestive enzyme activity. In vitro studies using Jian carp intestinal cells demonstrated that 4 mmol/L glutamine increased antioxidant capacity. Applications of glutamine-containing dipeptides in fish have shown that 0.36% Ala-Gln in Jian carp diets significantly improved growth, feed utilization, and muscle protein content, while 0.75% Ala-Gln supplementation in larval taimen (*Hucho taimen*) diets enhanced growth performance and antioxidant capacity.

1.2 Arginine

Beyond its role as an essential precursor for protein synthesis, arginine and its metabolites (ornithine, citrulline, and nitric oxide) play important roles in immune regulation and maintenance of intestinal mucosal structure and function. In human and terrestrial animal studies, arginine is recognized as an essential nutrient for tissue repair and an immunonutrient that serves as the primary amino acid precursor for polyamines required for intestinal repair. Oral administration of 2% arginine solution ameliorated ischemia-induced intestinal mucosal injury in rats, while dietary supplementation with 0.7% and 0.6% arginine improved microvillus development and overall growth performance, health status, and intestinal function in weaned piglets, respectively. These benefits likely arise from arginine' s ability to promote proliferation and growth of intestinal mucosal cells, thereby strengthening the intestinal mechanical barrier, reducing disease-induced intestinal damage, maintaining environmental stability, and ensuring mucosal integrity. However, arginine exhibits a "double-edged sword" effect: at "intestinal physiological levels" of 4 mmol/L, it benefits cell migration, whereas doses exceeding 10 mmol/L become detrimental. In fish, arginine is an essential amino acid, though reports on its effects on intestinal function remain limited. Dietary supplementation with 1% arginine and 1% glutamine improved intestinal function in red drum (*Sciaenops ocellatus*), while 1.85% arginine in Jian carp diets reduced lipopolysaccharide-induced intestinal damage.

The mechanisms underlying arginine' s role in intestinal repair are generally believed to involve promoting reactive oxygen species production and enhancing nitrotyrosylation of intestinal mucosa, making it a potent stimulator of enterocyte migration and epithelial recovery. Arginine also increases cell migration rates and activates downstream ribosomal protein S6 kinase 1 (S6K1) of the mammalian target of rapamycin (mTOR) pathway.

1.3 Other Amino Acids

Threonine is a major component of plasma -globulin and intestinal mucin, with approximately 70% of threonine intake metabolized by intestinal tissue in infants. Threonine deficiency in piglets reduces mast cells and goblet cell numbers in intestinal tissue while significantly decreasing intestinal mucin content, effects that cannot be fully reversed by intravenous threonine supplementation. Restricting dietary threonine significantly reduces mucin synthesis capacity in all segments of rat small intestine, while supplementation with 0.89% threonine improves intestinal function in piglets. During inflammatory responses, threonine deficiency weakens intestinal barrier function, whereas increased threonine supply promotes mucin synthesis and mucosal function recovery.

Leucine is considered a functional amino acid important for intestinal function, with 42-48% of infant leucine intake utilized by intestinal tissue compared to only 20-30% in adults. Lysine is also used for intestinal mucosal protein synthesis and can be catabolized to provide energy for the intestine, with 35% of dietary lysine sequestered by piglet intestine, of which only 18% is used for mucosal protein synthesis. In fish studies, appropriate tryptophan levels improved immune status, antioxidant capacity, and barrier structure integrity in the anterior, mid, and posterior intestine of mid-term grass carp (*Ctenopharyngodon idella*). Microencapsulated threonine effectively improved intestinal health and nutrient digestion and absorption capacity in juvenile Jian carp.

2 Mineral and Lipid Additives

2.1 Zinc

Zinc is a crucial factor affecting intestinal cell division and regeneration and regulating intestinal amino acid and protein metabolism. Dietary supplementation with 3,000 mg/kg zinc in weaned piglets promotes intestinal development and reduces stem cell factor mRNA and protein expression in the jejunum, thereby preventing enteritis. Zinc oxide supplementation enhances antioxidant capacity, inhibits intestinal cell apoptosis, and prevents enteritis in newborn piglets. In juvenile Jian carp, appropriate dietary zinc levels promote intestinal development, increase activities of digestive and brush border enzymes, and improve nutrient digestion and absorption capacity, thereby enhancing production performance.

2.2 Fatty Acids

Polyunsaturated fatty acids (PUFAs) have been confirmed beneficial for enteritis prevention and treatment. Dietary C18:3n-3 reduces inflammatory responses in young mice with colitis, while n-3 PUFAs decrease the incidence of necrotizing enterocolitis in mice. Eicosapentaenoic acid (EPA) added to human colon adenocarcinoma cell cultures affects tight junctions and permeability of intestinal monolayers, suggesting that n-3 PUFAs may prevent enteritis by enhancing intestinal barrier function. Free fatty acids added to porcine intestinal mucosa primary cultures promote development of brush border lipid raft microdomains. Dietary supplementation with 0.3% mixed medium-chain fatty acids in piglets affects gastric microbial populations and intestinal bacterial metabolite production, likely due to their immunoenhancing and antibacterial properties. In aquaculture, 0.02% plant essential oil improved growth performance and intestinal health in Pacific white shrimp (*Litopenaeus vannamei*) fed low-fishmeal diets.

2.3 Probiotics

Probiotics interact with various cellular components in the intestine and influence enterocyte function through multiple mechanisms. Major cellular signaling pathways and cytokines—including nuclear transcription factors, MAPKs, heat shock proteins, and peroxisome proliferator-activated receptors—are targets of probiotics or their products, which can modify and regulate these pathways in diverse ways. Probiotics are widely applied in aquaculture, where they have been shown to promote growth by enhancing intestinal digestive enzyme activities, maintaining bacterial balance, and strengthening immune capacity.

2.4 Carbohydrates

Carbohydrates that improve intestinal function include oligosaccharides and polysaccharides. Intraperitoneal injection of peptidoglycan significantly inhibited colon cancer cell growth in tumor-bearing mice. Plant oligosaccharides regulate intestinal microflora in livestock and poultry by inhibiting proliferation of harmful microorganisms while promoting *Bifidobacterium* and *Lactobacillus* growth. Oligosaccharides modulate intestinal microbiota primarily by proliferating beneficial bacteria (e.g., *Bifidobacterium*), inhibiting pathogens (e.g., *E. coli*), and preventing pathogen colonization to facilitate their elimination. Polysaccharides primarily enhance intestinal immune function to resist pathogen invasion by maintaining normal microcirculation, promoting proliferation of mucosal immune cells, improving antioxidant capacity, and regulating cytokine and inflammatory mediator secretion. In tilapia, dietary astragalus polysaccharide increased villus length and the numbers of intestinal goblet cells and intraepithelial lymphocytes.

3 Conclusion

In summary, intestinal health is a key factor ensuring rapid growth in aquatic animals. Various additives can promote intestinal development, maintain normal structure and function, and enhance nutrient transport and absorption capacity. Therefore, incorporating intestinal health-improving additives in aquafeeds can increase utilization of relatively inexpensive feed sources. However, the mechanisms by which these additives enhance digestion and absorption capacity in aquatic animals require further investigation.

References

[1] ZHOU X Q. Relationship between nutrients and fish intestinal health [C]// Advances in Animal Nutrition (2012 Edition). Beijing: Chinese Association of Animal Science and Veterinary Medicine, Animal Nutrition Branch, 2012: 246-260. [2] YU B, ZHANG K Y, ZHENG P, et al. Pig nutrition and intestinal health [J]. Chinese Journal of Animal Science, 2010, 46(15): 73-76. [3] GU M. Study on antinutritional factors and methionine affecting utilization of plant protein by marine fish and shrimp [D]. PhD Thesis. Qingdao: Ocean University

of China, 2013: 14. [4] WU G Y. Functional amino acids in growth, reproduction, and health [J]. Advances Nutrition: An International Review Journal, 2010, 1(1): 31-37. [5] CURI R, LAGRANHA C J, DOI S Q, et al. Molecular mechanisms of glutamine action [J]. Journal of Cellular Physiology, 2005, 204(2): 392-401. [6] XI P B, JIANG Z Y, ZHENG C T, et al. Regulation of protein metabolism by glutamine: implications nutrition health [J]. Frontiers Bioscience, 2011, 16(2): 578-597. [7] MARC R J, WU G Y. Glutamine, arginine, and leucine signaling in the intestine [J]. Amino Acids, 2009, 37(1): 111-122. [8] DAI Z L, LI X L, XI P B, et al. L-Glutamine regulates amino acid utilization by intestinal bacteria [J]. Amino Acids, 2013, 45(3): 501-512. [9] YI G F, CARROLL J A, ALLEE G L, et al. Effect of glutamine and spray-dried plasma on growth performance, small intestinal morphology, and immune responses of *Escherichia coli* K88+-challenged weaned pigs [J]. Journal of Animal Science, 2005, 83(3): 634-643. [10] CHEN G, SHI J, QI M, et al. Glutamine decreases intestinal nuclear factor kappa B activity and pro-inflammatory cytokine expression after traumatic brain injury in rats [J]. Inflammation Research, 2008, 57(2): 57-64. [11] ZHOU Y X, ZHANG P S, DENG G C, et al. Improvements of immune status, intestinal integrity and gain performance in the early-weaned calves parenterally supplemented with L-alanyl-L-glutamine dipeptide [J]. Veterinary Immunology Immunopathology, 2012, 145(1/2): 134-142. [12] UENO P M, ORIÁ R B, MAIER E A, et al. Alanyl-glutamine promotes intestinal epithelial cell homeostasis in vitro and in a murine model of weanling undernutrition [J]. American Journal of Physiology Gastrointestinal and Liver Physiology, 2011, 301(4): G612-G622. [13] HAYNES T E, LI P, LI X L, et al. L-Glutamine or L-alanyl-L-glutamine prevents oxidant- or endotoxin-induced death of neonatal enterocytes [J]. Amino Acids, 2009, 37(1): 131-142. [14] BRAGA-NETO M B, OLIVEIRA B M C, RODRIGUES R S, et al. Protective effects of alanyl-glutamine supplementation against nelfinavir-induced epithelial impairment in IEC-6 cells and in mouse intestinal mucosa [J]. Cancer Biology & Therapy, 2012, 13(14): 1482-1490. [15] AVISSAR N E, ZIEGLER T R, TOIA L, et al. ATB /ASCT2 expression in residual rabbit bowel decreased after massive enterectomy restored growth hormone treatment [J]. Journal of Nutrition, 2004, 134(9): 2173-2177. [16] POHLENZ C, BUENTELLO A, BAKKE A M, et al. Free dietary glutamine improves intestinal morphology and increases enterocyte migration rates, but has limited effects on plasma amino profile growth performance channel catfish *Ictalurus punctatus* [J]. Aquaculture, 2012, 370/371: 32-39. [17] YAN L, ZHOU X Q. Dietary glutamine supplementation improves structure and function of intestine juvenile (*Cyprinus carpio* var. Jian) [J]. Aquaculture, 2006, 256(1/2/3/4): 389-394. [18] CHEN J, ZHOU X Q, FENG L, et al. Effects of glutamine on hydrogen peroxide-induced oxidative damage intestinal epithelial cells (*Cyprinus carpio* var. Jian) [J]. Aquaculture, 2009, 288(3/4): 285-289. [19] XU H, ZHENG W, CHEN X M, et al. Effects of alanyl-glutamine and -aminobutyric acid on growth, feed utilization and body composition of Jian carp [J]. Journal of South China Agricultural University, 2016, 37(2): 7-13. [20] XU Q Y, WANG C A, XU H, et al. Effects of alanyl-glutamine on growth and antioxidant capacity of larval taimen (*Hucho taimen*) [J]. Chinese

Journal of Animal Nutrition, 2009, 21(6): 1012-1017. [21] WU G Y, BAZER F W, DAVIS T A, et al. Important roles for the arginine family of amino acids in swine nutrition and production [J]. Livestock Science, 2008, 112(1/2): 8-22. [22] SUKHOTNIK I, HELOU H, MOGILNER J, et al. Oral arginine improves intestinal recovery following ischemia-reperfusion injury rat [J]. Pediatric Surgery International, 2005, 21(3): 191-196. [23] ZHAN Z F, OU D Y, PIAO X S, et al. Dietary arginine supplementation affects microvascular development small intestine early-weaned pigs [J]. Journal of Nutrition, 2008, 138(7): 1304-1307. [24] WU X, RUAN Z, GAO Y L, et al. Dietary supplementation with L-arginine N-carbamylglutamate enhances intestinal growth and heat shock protein-70 expression in weanling pigs fed a corn- and soybean meal-based diet [J]. Amino Acids, 2010, 39(3): 831-839. [25] RHOADS J M, CHEN W, GOOKIN J, et al. Arginine stimulates intestinal cell migration through a focal adhesion kinase dependent mechanism [J]. Gut, 2004, 53(4): 514-522. [26] CHENG Z Y, BUENTELLO A, GATLIN D M. Effects of dietary arginine and glutamine on growth performance, immune responses and intestinal structure of red drum, *Sciaenops ocellatus* [J]. Aquaculture, 2011, 319(1/2): 247-252. [27] JIANG J, SHI D, ZHOU X Q, et al. In vitro and in vivo protective effect of arginine against lipopolysaccharide induced inflammatory response in the intestine of juvenile Jian carp (*Cyprinus carpio* var. Jian) [J]. Fish & Shellfish Immunology, 2015, 42(2): 457-464. [28] HOERR R A, MATTHEWS D E, BIER D M, et al. Effects of protein restriction and acute refeeding on leucine and lysine kinetics in young men [J]. American Journal of Physiology, 1993, 264(4 Pt 1): E567-E575. [29] BERTOLO R F, CHEN C Z, LAW G, et al. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically [J]. The Journal of Nutrition, 1998, 128(10): 1752-1759. [30] FAURE M, MOËNNOZ D F, MONTIGON F, et al. Dietary threonine restriction specifically reduces intestinal Mucin synthesis in rats [J]. The Journal of Nutrition, 2005, 135(3): 486-491. [31] WANG W W, ZENG X F, MAO X B, et al. Optimal dietary true ileal digestible threonine for supporting the mucosal barrier in small intestine of weanling pigs [J]. Journal of Nutrition, 2010, 140(5): 981-986. [32] WANG X, QIAO S Y, YIN Y L, et al. A deficiency or excess of dietary threonine reduces protein synthesis jejunum and skeletal muscle of young pigs [J]. Journal of Nutrition, 2007, 137(6): 1442-1446. [33] RIEDIJK M A, VAN GOUDOEVER J B. Splanchnic metabolism of ingested amino acids in neonates [J]. Current Opinion in Clinical Nutrition and Metabolic Care, 2007, 10(1): 58-62. [34] STOLL B, HENRY J, REEDS P J, et al. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets [J]. Journal of Nutrition, 1998, 128(3): 606-614. [35] WEN H L, FENG L, JIANG W D, et al. Dietary tryptophan modulates intestinal immune response, barrier function, antioxidant status and gene expression of TOR and Nrf2 in young grass carp (*Ctenopharyngodon idella*) [J]. Fish & Shellfish Immunology, 2014, 40(1): 275-287. [36] FENG L, PENG Y, LIU Y, et al. Comparative study on effects of crystalline threonine and microencapsulated threonine on growth performance and digestion and absorption capacity of juvenile Jian carp [J]. Chinese Journal of Animal Nutrition,

2011, 23(5): 771-780. [37] LI X L, YIN J D, LI D F, et al. Dietary supplementation with zinc oxide increases IGF-I and IGF-I receptor gene expression in the small intestine of weanling piglets [J]. The Journal of Nutrition, 2006, 136(7): 1786-1791. [38] OU D Y, LI D F, CAO Y H, et al. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs [J]. Journal of Nutritional Biochemistry, 2007, 18(12): 820-826. [39] WANG X Q, OU D Y, YIN J D, et al. Proteomic analysis reveals altered expression of proteins related to glutathione metabolism and apoptosis in the small intestine of oxide-supplemented piglets [J]. Amino Acids, 2009, 37(1): 209-218. [40] TAN L N. Effects of zinc on digestion and absorption capacity, immune function and antioxidant function in juvenile Jian carp [D]. Master's Thesis. Chengdu: Sichuan Agricultural University, 2009. [41] JACOBSON K, MUNDRA H, INNIS S M. Intestinal responsiveness to experimental colitis in young rats is altered by maternal diet [J]. American Journal of Physiology Gastrointestinal & Liver Physiology, 2005, 289(1): G13-G20. [42] CAPLAN M S, JILLING T. The role of polyunsaturated fatty acid supplementation in intestinal inflammation neonatal necrotizing enterocolitis [J]. Lipids, 2001, 36(9): 1053-1057. [43] USAMI M, MURAKI K, IWAMOTO M, et al. Effect of eicosapentaenoic acid (EPA) on tight junction permeability in intestinal monolayer cells [J]. Clinical Nutrition, 2001, 20(4): 351-359. [44] DE QUELEN F, CHEVALIER J, ROLLI-DERKINDEREN M, et al. n-3 polyunsaturated fatty acids in the maternal diet modify the postnatal development of nervous regulation of intestinal permeability in piglets [J]. Journal of Physiology, 2011, 589(17): 4341-4352. [45] HANSEN G H, RASMUSSEN K, NIELS-CHRISTIANSEN L L, et al. Dietary free fatty acids form alkaline phosphatase-enriched microdomains intestinal brush border membrane [J]. Molecular Membrane Biology, 2011, 28(2): 136-144. [46] ZENTEK J, BUCHHEIT-RENKO S, MÄNNER K, et al. Intestinal concentrations of free and encapsulated dietary medium-chain fatty acids and effects on gastric microbial ecology and bacterial metabolic products in the digestive tract of piglets [J]. Archives of Animal Nutrition, 2012, 66(1): 14-26. [47] WANG M Q, HUANG X L, JIN M, et al. Improvement effects of dietary plant essential oil on growth performance and intestinal health of *Litopenaeus vannamei* [J]. Chinese Journal of Animal Nutrition, 2015, 27(4): 1163-1171. [48] LIANG Y, QIN H L. Research progress on probiotics regulating intestinal cell signaling pathways [J]. Chinese Journal of Clinical Nutrition, 2012, 20(2): 112-116. [49] XIA L, ZHAO M J, ZHANG H Y, et al. Effects of different proportions of compound probiotics on growth, immunity and ammonia nitrogen resistance of *Litopenaeus vannamei* [J]. Journal of Fishery Sciences of China, 2015, 22(6): 1299-1307. [50] HU Y, TAN B P, MAI K S, et al. Effects of dietary probiotics on growth, intestinal microflora and partial immune indices of *Litopenaeus vannamei* [J]. Journal of Fishery Sciences of China, 2008, 15(2): 244-251. [51] MA X Y, LE G W, SHI Y H, et al. Inhibitory effect of *Lactobacillus* peptidoglycan on colon cancer cells and its immune mechanism [J]. Acta Nutrimenta Sinica, 2004, 26(6): 467-470. [52] WANG X W, DU Y G, BAI X F, et al. Effects of chitosan oligosaccharide on intestinal microflora, small intestinal microvillus density, immune function and production performance of broilers [J]. Chinese

Journal of Animal Nutrition, 2003, 15(4): 32-35. [53] SHENG Q K, YAO H Y. Regulatory mechanism of oligosaccharides on intestinal flora [J]. Animal Science and Animal Medicine, 2002, 19(2): 35-38. [54] HAN L L, WANG J F, WANG F L, et al. Research progress on effects of astragalus polysaccharide on intestinal immune function [J]. China Animal Husbandry & Veterinary Medicine, 2009, 36(8): 133-135. [55] HUANG Y Z, LIN X, WANG Q X, et al. Effects of astragalus polysaccharide on intestinal villus morphology and intestinal immune cells of tilapia [J]. Chinese Journal of Animal Nutrition, 2010, 22(1): 108-116.

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