Recent Advancements in Nutrition and Health Interactions Mediated by the Gastrointestinal Tract

Delbert M. Gatlin III*

Department of Wildlife and Fisheries Sciences and Intercollegiate Faculty of Nutrition, Texas A&M University, College Station, TX 77840, USA

*Correspondence to: D.M. Gatlin, 216 Heep Laboratory Building, 2258 TAMUS, College Station, TX 77843, USA. Tel. +1-979-847-9333, fax +1-979-845-4096, E-mail d-gatlin@tamu.edu

Abstract

Diet additives that influence immunity and disease resistance of aquatic species continue to receive considerable attention due their potential to positively influence intensive aquaculture. Prebiotics, probiotics and essential oils are some of the prominent types of feed additives that may influence the microbiota of the gastrointestinal tract (GIT). It has been documented that microbiota of the GIT play important roles in affecting the nutrition and health of the host organism. Thus, various means of altering the intestinal microbiota to achieve favorable effects such as enhancing growth, digestion, immunity and disease resistance of the host organism continue to be pursued.

The first line of defense within the GIT is the mucosa that separates the gut microbiota from direct contact with the epithelial cells of the GIT. It is because of this direct contact with the mucus that the immune system of the GIT, often referred to as gut-associated lymphoid tissue or GALT, has developed mechanisms to distinguish between potentially pathogenic bacteria and the normal, commensal bacteria. In the event that potentially pathogen bacteria are detected, the cellular and humoral mechanisms of the GALT activate the innate immune system and subsequently the adaptive immune system to prevent bacteria from causing and/or spreading infection. It is anticipated that continued advancements with diet additives that influence the GIT will allow nutritional modulation of immunity to be increasingly used as an effective and relatively inexpensive means of combating diseases in aquaculture.

Keywords: immunonutrition, essential oils, feed additives, fish health, immune responses, prebiotics, probiotics

Gatlin, D. 2015. Recent Advancements in Nutrition and Health Interactions Mediated by the Gastrointestinal Tract. En: Cruz-Suárez, L.E., Ricque-Marie, D., Tapia-Salazar, M., Nieto-López, M.G., Villarreal-Cavazos, D. A., Gamboa-Delgado, J., Rivas Vega, M. y Miranda Baeza, A. (Eds), Nutrición Acuícola: Investigación y Desarrollo, Universidad Autónoma de Nuevo León, San Nicolás de los Garza, Nuevo León, México, pp. 274-286.

Introduction

It is well established that proper nutrition is critical not only to achieve optimal growth rates but also to maintain adequate health of cultured fish (NRC 2011). In recent years, the influence of nutrition not only on normal growth but also its role in health management through modulation of immune response and disease resistance has received heightened attention. The targeted goal of this research has been to lessen the dependence on chemotherapeutics and reduce disease-related losses (Kiron, 2012; Oliva-Teles, 2012). Some have referred to this research area as "immunonutrition", which may be defined as the study of enhancing immunological functions by means of using specific nutrients and/or other dietary compounds. It has become apparent in studying various nutrients and diet additives that the microbiota of the gastrointestinal tract (GIT) plays important roles in affecting the nutrition and health of the host organism. Thus, various means of altering the intestinal microbiota to achieve favorable effects such as enhancing growth, digestion, immunity and disease resistance of the host organism have been increasingly investigated in recent years. In particular, diet additives that affect the GIT such as probiotics, prebiotics, and essential oils have been evaluated with growing intensity, as have certain nutrients which are involved in specific functions in the GIT such as arginine and glutamine.

The aim of the present review is to highlight the importance of the GIT in maintaining fish health, and the influence of specific diet additives that can positively influence the GIT to enhance its functions for maintaining health and protecting against disease-causing organisms. Particular attention will be given to how the GIT mediates various immunological responses, and some of the prominent diet additives that influence the GIT either by influencing its microbiota or enhancing its immunocompetence.

Discussion

The Gastrointestinal Tract Microbiota and Their Numerous Functions

It has become increasingly apparent in recent years that the microbiota of the fish's GIT may influence a wide variety of metabolic processes (Dubert-Ferradon, *et al*.,2008). This microbiota stimulates epithelial proliferation and expression of numerous genes. Prominent functions among these are various physiological, biochemical, and immunological responses that must be maintained or enhanced to improve health status, stress responses and disease resistance. In addition, there are various other responses mediated by the GIT that may synergistically enhance weight gain and feed utilization of the cultured organism. The important functions of the GIT microbiota in relation to the immune system are of particular relevance in terms of maintaining fish health.

The Gastrointestinal Tract and Immune Systems

The role of the GIT in maintaining fish health is an area that has continued to expand over the past decade. The first line of defense within the GIT is the mucosa that separates the gut microbiota from direct contact with the epithelial cells of the GIT (Dubert-Ferrandon *et al*., 2008; Pérez *et al*., 2010). Due to this direct contact with the mucus, the immune system of the GIT, also referred to as gut-associated lymphoid tissue or GALT, has developed mechanisms to distinguish between potentially pathogenic bacteria and the normal, commensal autochthonous bacteria. As a result, the GALT may determine whether to mount an attack or tolerate a specific bacteria's presence (Pérez *et al*., 2010). In the event that potentially pathogen bacteria are detected, various cellular and humoral mechanisms of the GALT activate the innate immune system and subsequently the adaptive immune system to prevent bacteria from causing and/or spreading infection (Gomez and Balcázar, 2008). There are various components of the innate or non-specific immune response which can be activated to kill invading pathogens including such factors as blood neutrophil oxidative radical production, serum lysozyme and superoxide anion production in activated macrophages (Nayak, 2010). The purpose of these factors is to kill a wide variety of foreign or invading microorganisms, and their enhancement may lead to significant reductions in mortality of the host when exposed to various pathogenic organisms.

The adaptive immune system is more complex than the innate immune system but is activated and to some extent directed by the innate immune system. Components of the adaptive or specific immune system include lymphocytes such as B cells and T cells which allow the host to recognize and combat specific disease-causing organisms. The adaptive immune system allows vertebrates, including fish, to recognize and remember specific pathogens and generate immunity against future exposure to such pathogens. This complex part of the immune system has not been studied extensively in response to supplementation of various diet additives but some of its components appear to be enhanced. Thus, it is recommended that additional research in this area be conducted to more fully characterize the effects of additives such as probiotics, prebiotics and essential oils on adaptive immunity of the host organism.

Diet Additives that Influence Microbiota of the GIT

The intestinal microbial populations are composed of two primary groups: those that are permanent colonizers (autochthonous bacteria), and transients (allochthonous bacteria). The autochthonous bacteria are resident populations which colonize the epithelial surface of the host organism's GIT, including the microvilli. These health-promoting bacteria, such as lactobacilli, may provide a defensive barrier and protect against the invasion of bacterial pathogens via the GIT. It is this group of bacteria that is generally targeted for manipulation by prebiotics and probiotics (Ringo *et al*., 2010). Establishment of bacterial pathogens in the GIT also may be impeded by the mucus layer of the GIT, which provides physical as well as various types of biochemical protection. The GIT is among the most common sites of pathogen entrance in fish, because they are exposed to water which contains various types of potentially pathogenic bacteria. However, a healthy gut microbiota has the ability to prevent pathogenic bacteria from colonizing the intestine, thus preventing infection (Birkbeck and Ringø, 2005; Stecher and Hardt, 2008). The autochthonous bacteria of the GIT which are present under normal conditions act to competitively exclude pathogens simply by their presence. By taking up space and resources along the mucosal lining of the GIT, pathogenic bacteria are forced to continue in a transient state where the likelihood of damaging intestinal cells or causing infection is reduced. Autochthonous bacteria also have the capacity to produce antimicrobial substances, which enhance their ability to inhibit pathogens from attempting to colonize the GIT. However, when the natural equilibrium state of the microbiota is altered, conditions become more favorable for pathogenic organisms to flourish.

There is one broad group of diet additives that has been investigated with terrestrial and aquatic organisms which influence the microbiota of the GIT. These additives include prebiotics, probiotics and essential oils. Prebiotics are defined as non-digestible food ingredients which beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of one or a limited number of health-promoting bacteria in the GIT, thus improving the host's intestinal balance (Gibson and Roberfroid, 1995). The health-promoting bacteria most commonly augmented by prebiotics include those of the genus *Lactobacillus* which tend to limit the presence of harmful bacteria. Many prebiotics are oligosaccharides composed of various sugars while other ingredients shown to possess prebiotic properties include GroBiotic®-A, a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products. The chemical nature and ways in which various prebiotic compounds may affect the host organism have been summarized by Gatlin and Peredo (2012). Prebiotics are not typically altered by diet processing and require limited regulatory approval such that their incorporation into diets is much simpler than required for chemical therapeutic agents.

Probiotics are live microbial additives that may alter microbiota of the GIT. The term probiotic was introduced by Parker (1974) as "organisms and substances which contribute to intestinal microbial balance". Fuller (1989) revised the definition as "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Subsequently, Moriarty (1998) proposed that the definition of probiotics be extended to microbial “water additives”. Administration of probiotics to the water has been associated with improving water quality such as reductions in the concentrations of nitrogen and phosphorus via microbial transformations (Wang *et al*., 2005). In addition to affecting water quality, probiotics administered to the water or diet also may inhibit the growth of pathogenic microorganisms, contribute digestive enzymes which may increase feed utilization, provide other growth-promoting factors as well as stimulate the immune response of the host organism.

Probiotics that have been shown to influence fish immunity, disease resistance and other performance indices include those of the genus *Bacillus* and various lactic acid bacteria (*Lactobacillus, Lactococcus, Carnobacterium, Pediococcus, Enterococcus* and *Streptococcus*). Bacteria of the genus *Bacillus* are Gram-positive rods that form spores and are resistant to environmental conditions and thus have extended shelf life. *Lactobacillus rhamnosus, L. delbrüeckii, Carnobacterium maltaromaticum, C. divergens, C. inhibens,* and *Enterococcus faecium* are other bacterial species which have been used as probiotics, as well as yeasts such as *Saccharomyces cerevisiae* and *Candida sake*. Viability of the bacteria during storage and processing is critical for probiotics to confer their beneficial effects to the host organism, however, the application of dead cells, lyophilized cells or cell-free supernatants or spores have all showed some degree of success (Merrifield *et al*., 2010). The logistical complication of culturing live microorganisms prior to application on the feed has constrained the use of probiotics at aquaculture facilities and thus administration of lyophilized cells or spores may be more practical. Potential applications of probiotics in fish, shrimp and molluscan aquaculture have been reviewed by Burr *et al*. (2005), Wang *et al*. (2008), Kesarcodi-Watson *et al*. (2008) and most recently by Ringø *et al*. (2010).

In order to help maintain the delicate balance between microbiota of the GIT, prebiotics or probiotics may be included in the diet to help reinforce the population of beneficial bacteria while decreasing the number of potentially pathogenic bacteria. Probiotics may directly accomplish this by providing an increased number of desirable bacteria when incorporated in the diet and ingested. A regular supply of beneficial bacteria added to the GIT will help to control or reduce the number of detrimental bacteria. In the case of prebiotics, they accomplish their goal more indirectly, by acting as a food source, preferentially, to the beneficial bacteria. It also has been shown that some pathogenic bacteria may become bound to certain prebiotics, as opposed to attaching to the mucosal lining of the GIT, and thereby may be passed from the GIT (Spring *et al*., 2000).

Another group of diet additives that may influence the microbiota of the GIT and is receiving a growing amount of attention in recent years is referred to essential oils. These essential oils are obtained from plants such as basil, cinnamon, and oregano, and may contain a rich mixture of highly functional molecules with a wide spectrum of biological activity. Some studies have demonstrated that these essential oils when added to diets can activate the fish antioxidant defense system, enhance various immune functions, change the intestinal morphology and microbiota, as well as increase digestibility and nutrient absorption (e.g., Harikrishnan *et al*., 2011; Giannenas *et al*., 2012; Saccol *et al*., 2013).

Practical application of prebiotics, probiotics and essential oils

As mentioned previously, maintaining the viability of probiotics during storage and processing is important for them to exert their beneficial effects to the host organism. Therefore, some logistical constraints may be encountered with the cultivation of live microorganisms in conjunction with manufacturing feeds. To ensure probiotic viability, its application to the feed typically must occur post extrusion so the probiotic organism is not subjected to excessive heat and pressure. As such, administration of probiotics in the form of lyophilized cells or spores may be less demanding compared to live organisms. In general, feed manufacturing constraints are of less concern when dealing with prebiotics because they are not living organisms. Although the efficacy of several prebiotics has been shown when incorporated into extrusion-processed feeds, the potential chemical alteration of prebiotic compounds during feed manufacturing has not be studied to any appreciable extent and deserves further consideration. Similar feed manufacturing issues also have not been thoroughly addressed with various essetial oil compounds but deserve further evaluation.

Administration regimes for specific prebiotics, probiotics and essential oils also have not been widely studied to date. Although these compounds have immunostimulating effects, it does not appear that long-term administration causes immunosuppression as noted with other potent immunostimulants such as beta glucans. Therefore, it is generally assumed that these diet additives may be administered for extended periods. However, developing more refined administration protocols for individual diet additives should be investigated to optimize their effectiveness. For example, administering probiotics, prebiotics or essential oils at prescribed times before the culture organism is exposed to a stressful event or at particular times of the year when pathogenic organisms are most prevalent could possibly be developed to most efficiently derive the benefits of these compounds under particular culture regimes.

Nutrients of the Immune System

Another type of immunonutrition which has been investigated to a limited extent is the dietary supplementation of essential nutrients at levels above which are required for normal physiological functions and which are intended to specifically nourish the immune system. In quiescent immune cells, the utilization of nutrients happens at basal levels, merely for cellular maintenance. However, during an immune challenge, the utilization of key nutrients dramatically increases. In particular, there is an important demand for amino acids (Kiron, 2012; Uribe *et al*., 2011). For example, *in vivo* reports suggest important usage of glutamine in ill fish, as reflected by the rapid decrease in plasma levels of this amino acid (Walker *et al*., 1996). Likewise, arginine is the unique precursor of nitric oxide in activated macrophages (Buentello and Gatlin, 1999, Neumann *et al*., 1995; Wu and Morris, 1998), and is a potent microbicidal compound and potent modulator of the eukaryotic cytoskeleton (Kasprowicz *et al*., 2009; Moffat *et al*., 1996). Although the profile of the amino acids used will vary depending on if there is phagocytic or lymphocytic responses, arginine and glutamine both have been demonstrated to play key roles in the overall performance of these immune cells of fish (e.g., Pohlenz *et al*., 2012a), and supplementation in the diet may achieve favorable effects. Supplementing diets with nutrients that could be metabolically used by phagocytes has been an area of interest for enhancing that stage of the immune response. For instance, glutamine has been shown to provide metabolic fuel to support reaction kinetics (Crawford and Cohen, 1985; Newsholme and Newsholme, 1989).

Exogenous sources of nutrients should supply minimum levels to meet requirements for normal immune system performance and to protect/restore tissues from collateral damage. However, in certain situations, providing additional nutrient at levels above those required for normal fish maintenance and growth may sustain and/or enhance one or more functions of the immune system, thereby increasing its efficacy and protection capacity against invading pathogens (Kiron 2012; Sealey and Gatlin 2001). Dietary supplementation of both arginine and glutamine was able to enhance vaccination efficiency of channel catfish *Ictalurus punctatus* (Pohlenz *et al*., 2012b).

Conclusions

It can be seen from this review that various nutrients and diet additives can positively affect the health and immune responses of fish through either modulation of the microbiota of the GIT or specifically nourishing immune cells. Nutritional modulation of the immune system continues to be a potentially powerful tool to improve the health and growth of cultured fish, and hence to improve production efficiency and yield. However, because of the great diversity of fish being cultured along with a lack of full understanding regarding the fish's immune system, immunonutrition is still not fully developed in aquaculture. This area does warrant additional research in order to reach its full potential and application. In particular, there is a considerable need to fine-tune dosing of various additives, optimize feeding regimes, and supplementation strategies so that immunonutrition can become more effective and used efficiently to benefit the production of various aquatic species.

References

Birkbeck T. H., Ringø E. (2005) Pathogenesis and the gastrointestinal tract of growing fish.In: *Microbial Ecology in Growing Animals* (ed.by W. Holzapfel and P. Naughton), pp. 208-234. Elsevier, Edinburgh, UK.

Buentello J.A., Gatlin D.M. (1999) Nitric oxide production in activated macrophages from channel catfish (*Ictalurus punctatus*): influence of dietary arginine and culture media. *Aquaculture* **179**, 513-521.

Burr G., Gatlin D.M., III, Ricke S. (2005) Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in finfish aquaculture. *Journal of the World Aquaculture Society* **36**, 425-436.

Crawford J., Cohen H.J. (1985) The essential role of L-glutamine in lymphocyte differentiation *in vitro*. *Journal of Cell Physiology* **124**, 275-282.

Dubert-Ferradon A., Newburg D.S., Walker A.W. (2008) Immune functions and mechanisms in the gastrointestinal tract. In: *Handbook of Prebiotics* (ed. G.R. Gibson and M.B. Roberfroid), pp. 115-134. CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA.

Fuller R., (1989) Probiotics in man and animals. *Journal of Applied Bacteriology* **66**, 365-378.

Gatlin D.M., III, Peredo, A. (2012) *Prebiotics and Probiotics: Definitions and Applications,* SRAC Publication No. 4711, 8 pp.

Giannenas I., Triantafillou E., Stavrakakis S., Margaroni M., Mavridis S., Steiner T., Karagouni E. (2012) Assessment of dietary supplementation with carvacrol or thymol containing feed additives on performance, intestinal microbiota and antioxidant status of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **350-353**, 26-32.

Gibson G.R., Roberfroid M.B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition* **125**, 1401-1412.

GÓmez G.D., Balcázar J.L. (2008) A review on the interactions between gut microbiota and innate immunity of fish. FEMS *Immunology and Medical Microbiology* **52**, 145-154.

Harikrishnan R., Balasundaram C., Heo M. (2011) Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture* **317**, 1-15.

Kasprowicz A., Szuba A., Volkmann D., Baluska F., Wojtaszek P. (2009) Nitric oxide modulates dynamic actin cytoskeleton and vesicle trafficking in a cell type-specific manner in root apices. *Journal of Experimental Botony* **60**, 1605-1617.

Kesarcodi-Watson A., Kaspar H., Lategan M.J., Gibson L. (2008) Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. *Aquaculture* **274**, 1-14.

Kiron V. (2012) Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology* **173**, 111-133.

Merrifield D.L., Dimitroglou A., Foey A., Davies S.J., Baker R.T.M., Bøgwald J., Castex M. and Ringø E. (2010) The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture* **302**, 1-18.

Moffat F.L., Jr., Han T., Li Z.M. Peck M.D., Jy W., Ahn Y.S., Chu A.J., Bourguignon L.Y. (1996) Supplemental L-arginine HCl augments bacterial phagocytosis in human polymorphonuclear leukocytes. *Journal of Cell Physiology* **168**, 26-33.

Moriarty D.J.W. (1998) Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture* **164**, 351-358.

Nayak S. K. (2010) Probiotics and immunity: a fish perspective. *Fish & Shellfish Immunology* 2**9**, 2-14.

Neumann N.F., Fagan D., Belosevic M. (1995) Macrophage activating factor(s) secreted by mitogen stimulated goldfish kidney leukocytes synergize with bacterial lipopolysaccharide to induce nitric oxide production in teleost macrophages. *Developmental and Comparative Immunology* **19**, 473-482.

Newsholme P., Newsholme E.A. (1989) Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. *Biochemical Journal* **261**, 211-218.

NRC (2011) *Nutrient Requirements of Fish and Shrimp*. The National Academies Press, Washington, D.C.

Oliva-Teles A. (2012) Nutrition and health of aquaculture fish. *Jornal of Fish Diseases* **35**, 83-108.

Parker R.B. (1974) Probiotics, the other half of the antibiotics story. *Animal Nutrition and Health* **29**, 4-8.

Pérez T., Balcázar J.L., Ruiz-Zarzuela I., Halaihel N., Vendrell D., de Blas I., Múzquiz J.L. (2010) Host-microbiota interactions within the fish intestinal ecosystem. *Mucosal Immunology* **4**, 355-360.

Pohlenz C., Buentello A., Mwangi W., Gatlin D.M., III (2012a) Arginine and glutamine supplementation to culture media improves the performance of various channel catfish immune cells. *Fish & Shellfish Immunology* **32**, 762-768.

Pohlenz C., Buentello A., Criscitiello M.F., Mwangi W., Smith R., Gatlin D.M., III (2012b) Synergies between vaccination and dietary arginine and glutamine supplementation improve the immune response of channel catfish against *Edwardsiella ictaluri*. *Fish & Shellfish Immunology* **33**, 543-551.

Ringo E., Lovmo L., Kristiansen M., Bakken Y., Salinas I., Myklebust R., Olsen R.E., Mayhew T.M. (2010). Lactic acid bacteria vs. pathogens in the gastrointestinal tract of fish: a review. *Aquaculture Research* **41**, 451-467.

Saccol E.M.H., Uczay J., Pês T.S., Finamor I.A., Ourique G.M., Riffel A.P.K., Schmidt D., Caron B.O., Heinzmann B.M., Llesuy S.F. Lazzari R., Baldisserotto B. Pavanato M.A. (2013) Addition of *Lippia alba* (Mill) N. E. Brown essential oil to the diet of the silver catfish: An analysis of growth, metabolic and blood parameters and the antioxidant response. *Aquaculture* **416-417**, 244-254.

Sealey W.M., Gatlin D.M. (2001) Overview of nutritional strategies affecting the health of marine fish. in: *Nutrition and Fish Health* (ed. C. Lim, C.D.Webster), pp. 103-118. Food Products Press, Binghamton, N.Y.

Spring P., Wenk C., Dawson K.A., Newman K.E. (2000) The effects of dietary mannanoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of Salmonella-challenged broiler chicks. *Poultry Science* **79**, 205-211.

Stecher B., Hardt W.-D. (2008) The role of microbiota in infectious disease. *Trends in Microbiology* **16**, 107-114.

Uribe C., Folch H., Enriquez R., Moran G. (2011) Innate and adaptive immunity in teleost fish: a review. *Veterinary Medicine* **56**, 486-503.

Walker S.P., Keast D., McBride S. (1996) Distribution of glutamine synthetase in the snapper (*Pagrus auratus*) and implications for the immune system. *Fish Physiology and Biochemistry* **15**, 187-194.

Wang Y.B., Xu Z.R., Xia M.S. (2005) The effectiveness of commercial probiotics in Northern White Shrimp (*Penaeus vannamei* L.) ponds, *Fisheries Science* **71**, 1034-1039.

Wang Y.-B., Li J.-R., Lin J. (2008) Probiotics in aquaculture: Challenges and outlook. *Aquaculture* **281**, 1-4.

Wu G., Morris S.M., Jr. (1998) Arginine metabolism: nitric oxide and beyond. *Biochemical Journal* **336**, 1-17.