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Prebiotics and Probiotics: Definitions and Applications

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Infectious diseases cost the aquaculture industry millions of dollars each year. Therefore, there is great interest in developing alternatives to traditional means of combating diseases with chemotherapeutics. Traditional disease prevention and treatment strategies, such as the use of vaccines and drugs, have limitations such as regulatory constraints or inconvenient means of delivery. This has heightened interest in the use of dietary additives that may influence the immunity and disease resistance of aquatic species. Although the concept of functional feeds is relatively new to the aquaculture industry, there is promise in developing diets that do not just satisfy minimum nutritional requirements but also improve health and resistance to stress and disease-causing organisms. Two types of feed additives that have been investigated with terrestrial and aquatic organisms are those which influence the microbiota of the gastrointestinal tract (GIT). The additives are broadly grouped as prebiotics and probiotics.

Prebiotics

Prebiotics are defined as non-digestible food ingredients that selectively stimulate the growth and/or the metabolism of health-promoting bacteria in the intestinal tract, thus improving an organism's intestinal balance (Gibson and Roberfroid, 1995). The health-promoting bacteria most commonly augmented by prebiotics include those of the genus *Lactobacillus* and *Bifidobacter,* which tend to limit the presence of harmful bacteria. Examples of prebiotics include mannanoligosaccharides, lactose, galactogluco-mannans, oligofructose, and inulin. Many of these prebiotics are carbohydrates, primarily short-chain oligosaccharides consisting of three to ten carbohydrate units, which are derived from various plants or cell wall components of yeast. A commercial product that possesses prebiotic properties is the yeast-based product GroBiotic®-A, which is a mixture of partially autolyzed brewers yeast, dairy ingredient components, and dried fermentation products. The various prebiotic compounds are generally not altered by diet processing and require limited regulatory approval, making their use much simpler than using drugs or chemical therapeutic agents. The benefits of prebiotics are described below and summarized in Table 1.

Probiotics

The term probiotic was defined 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." Administering probiotics in water has been shown to improve water quality by reducing the concentrations of nitrogen and phosphorus (Wang et al., 2005). Probiotics administered in water or diet also may inhibit the growth of pathogenic microorganisms, contribute digestive enzymes to increase feed utilization, provide other growth-promoting factors, and stimulate the immune response of the organism.

Recognized probiotics that may 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 that are resistant to various environmental condi-

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tions and thus have extended shelf life. *Bacillus subtillis*, *B. licheniformis*, *B. circulans, B. coagulans, B. clausii,* and *B. megaterium* all have been used as probiotics. *Lactobacillus rhamnosus*, *L. delbrüeckii*, *Carnobacterium maltaromaticum*, *C. divergens, C. inhibens,* and *Enterococcus faecium* are other bacteria that have been used as probiotics, along with yeasts such as *Candida sake* and *Saccharomyces cerevisiae*. The bacteria must remain viable during storage and processing for probiotics to confer their beneficial effects, but the application of dead cells, freeze-dried cells, or cell-free extracts or spores have all shown some degree of success (Merrifield et al., 2010). The logistical constraint of culturing live microorganisms under controlled laboratory conditions before applying them to the feed has constrained the use of probiotics at aquaculture facilities; thus, administering 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. (2010a).

Table 1. Summary of prebiotics evaluated in aquaculture (adapted from Ringø et al., 2010b).

Prebiotic[a]	Dose (g kg^{-1}); duration of trial	Species	Initial weight (g)	Response[b]	Reference
Inulin	150; 4 weeks	Arctic charr (*Salvelinus alpines*)	218	Intestinal cell damage	Olsen et al. (2001)
	75; 3 weeks	Atlantic salmon (*Salmo salar*)	172	→ Intestinal cell damage; ↑ Intestinal growth and relative mass of the gastrointestinal tract	Refstie et al. (2006)
	5 and 10; 1 week	Gilthead seabream (*Sparus aurata*)	175	Significant inhibition of phagocytosis and respiratory burst in leucocytes	Cerezuela et al. (2008)
	20; 1 month	Turbot larvae (*Psetta maxima*)	n/a	↑ Growth rate; Effects on gut microbiota (*Bacillus* and *Vibrio*)	Mahious et al. (2006)
MOS	10; 4 months	Atlantic salmon	200	↓ Oxygen consumption; ↓ Protein and ↑ energy concentration in the whole body	Grisdale-Helland et al. (2008)
	2; 4 weeks	Channel catfish (*Ictalurus punctatus*)	16.0	→ Growth performance, hematology, or immune function	Welker et al. (2007)
	20 and 40; 67 days	European sea bass (*Dicentrarchus labrax*)	33.7	↑ Weight gain (WG); → Feed efficiency (FE); ↓ Lipid vacuolization; ↓ Presence of *Vibrio alginolyticus* in head kidney	Torrecillas et al. (2007)
	2; 90 days	Rainbow trout (*Oncorhynchus mykiss*)	30.0	↑ WG and survival; ↑ Antibody titer and lysozyme activity	Staykov et al. (2007)
	0.2; 43 days	White seabream larvae (*Diplodous sargus*)	n/a	↑ Microvilli length	Dimitroglou et al. (2010)
	0 and 4; 12 weeks	Rainbow trout	13.2	↑ WG; ↑ Hemolytic and phagocytic activity; ↑ Mucus weight; ↑ Survival against *Vibrio anguillarum*	Rodrigues-Estrada et al. (2008)

Prebiotic[a]	Dose (g kg^{-1}); duration of trial	Species	Initial weight (g)	Response[b]	Reference
MOS (continued)	0, 2 and 6; 58 days	Hybrid tilapia (*Oreochromis niloticus* × *O. aureus*)	8.1	→ WG; ↑ Survival; ↑ Non-specific immunity	He et al. (2003)
	10; 4 weeks	Red drum (*Sciaenops ocellatus*)	10.9	↑ FE; ↑ Survival following parasitic challenge; ↑ Non-specific immunity	Buentello et al. (2010)
FOS	10; 4 months	Atlantic salmon	200	→ Feed intake, WG or digestibility	Grisdale-Helland et al. (2008)
	10; 4 weeks	Red drum	10.9	↑ Non-specific immunity	Buentello et al. (2010)
	0, 2 and 6; 58 days	Hybrid tilapia	57.0	→ WG; ↑ Survival; ↑ Non-specific immunity	He et al. (2003)
	20; 1 month	Turbot larvae	n/a	↑ WG; Effects on gut microbiota (*Bacillus* and *Vibrio*)	Mahious et al. (2006)
	20; 7 weeks	Beluga (*Huso huso*)	19.2	↑ Survival; Elevated lactic acid bacteria	Hoseinfar et al. (2011)
scFOS	0.8 and 1.2; 8 weeks	Hybrid tilapia	5.6	↑ WG, feed intake, FE; → Survival	Hui-Yuan et al. (2007)
	0.1 and 0.8; 6 weeks	White shrimp (*Litopenaeus vannamei*)	75.4	→ WG; → Survival; → FE; Altered microbial community	Li et al. (2007)
GBA	10 and 20; 4 (Trial 1) and 7 (Trial 2) weeks	Hybrid striped bass (*Morone chyrsops* × *M. saxatilis*)	91.4 (Trial 1) and 19.7 (Trial 2)	↑ FE; ↑ Respiratory burst; ↑ Resistance against *Streptococcus iniae*	Li and Gatlin (2004)
	20; 16 weeks	Hybrid striped bass	64.5	↑ Growth performance; ↑ Resistance against *Mycobacterium marinum*	Li and Gatlin (2005)
	10; 6 weeks	Red drum	2.4	→ WG or FE; → Intestinal microbiota	Burr et al. (2009)
	10; 4 weeks	Red drum	10.9	↑ FE; ↑ WG; ↑ Survival following parasitic challenge; ↑ Non-specific immunity	Buentello et al. (2010)
	20; 16 weeks	Golden shiner (*Notemigonus crysoleucas*)	1.06	↑ Resistance against *Flavobacterium columnare*	Sink et al. (2007)
	20; 10 weeks	Golden shiner	0.46	→ Survival; ↑ Resistance against *Flavobacterium columnare*	Sink and Lochmann (2008)
	10; 3 weeks	Red drum	500	↑ Protein, lipid and organic apparent digestibility coefficient values	Burr et al. (2008a)
	10 and 20; 8 weeks	Hybrid striped bass	34.4	→ WG or FE	Burr et al. (2010)

Prebiotic[a]	Dose (g kg^{-1}); duration of trial	Species	Initial weight (g)	Response[b]	Reference
GBA (continued)	20; 9	Rainbow trout	14.3	→ WG or FE; → Antibody levels	Sealey et al. (2007)
	4, 8 and 12; 8 weeks	Nile tilapia	18.0	↑ WG; ↑ FE; ↑ Neutrophil oxidative production; ↑ Lysozyme; ↑ Resistance against *A. hydrophila*	Zheng et al. (2011)
XOS	0, 0.15, 2.1 and 3.2; 45 days	Crucian carp (*Carassius auratus gibelio*)	17.0	↑ WG; → Survival; ↑ Enzymatic activity	Xu et al. (2009)
Galacto-gluco-mannan	10; 8 weeks	Red drum	7.0	↑ WG; → FE; ↑ Lysozyme; ↑ Microvillus height in pyloric caeca, proximal intestine and mid-intestine	Zhou et al. (2010)

[a] Prebiotics are abbreviated as follows: MOS = mannanoligosaccharides; FOS = fructooligosaccharides; scFOS = short-chain fructooligosaccharides; GBA = GroBiotic®-A; XOS = xylooligosaccharides.

[b] Arrows indicate an increase (↑), decrease (↓), or no change (→) in response. WG = Weight gain; FE = Feed efficiency.

Effects of gastrointestinal tract microbiota of fish

The intestinal microbiota are composed of two primary groups—those that are permanent colonizers (autochthonous bacteria) and transients (allochthonous bacteria). The autochthonous bacteria are resident populations that colonize the epithelial surface of the GIT, including the microvilli. These bacteria may provide a defensive barrier against the invasion of bacterial pathogens via the GIT. The establishment of bacterial pathogens in the GIT also may be impeded by the mucus layer, which provides physical and biochemical protection. In recent years it has become increasingly apparent that the microbiota of the GIT of fish may influence a wide variety of metabolic processes. This influence is mediated by the microbiota stimulating epithelial proliferation and expression of numerous genes. Prominent 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, various other responses may synergistically enhance weight gain and feed utilization of the cultured organism. The sections below highlight some of these important aspects and how they may be modulated by prebiotics and probiotics.

Pathogen entrance

The GIT is one of the most common sites of pathogen entrance in fish, given that fish are constantly exposed to water containing various types of potentially pathogenic bacteria. However, a healthy gut microbiota can prevent pathogenic bacteria from colonizing the intestine and thus causing infection. The autochthonous bacteria of the GIT, which are present under normal conditions, competitively exclude pathogens simply by taking up space and resources along the mucosal lining of the GIT, forcing pathogenic bacteria to continue in a transient state and lessening the likelihood that they will damage intestinal cells or cause infection. Autochthonous bacteria also can produce antimicrobial substances that help fend off pathogens 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.

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 accomplish this directly by introducing more desirable bacteria into the GIT. Prebiotics are beneficial because they act as a preferential food source for 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 thus may be passed from the GIT.

The immune system

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 autochthonous bacteria. Consequently, the GALT can determine whether to mount an attack or tolerate a specific bacteria's presence. If potentially pathogenic bacteria are detected, the cellular and humoral mechanisms of the GALT activate the innate immune system and, subsequently, the adaptive immune system (via antibodies) to prevent bacteria from causing and/or spreading infection (Gomez and Balcázar, 2008).

Components of the innate or non-specific immune response include such factors as blood neutrophil oxidative radical production, serum lysozyme, and superoxide anion production in activated macrophages. These various responses are intended to kill a wide variety of foreign or invading microorganisms, and enhancing them may significantly reduce the mortality of the aquatic organism when exposed to various pathogens (Table 1).

Adaptive immunity is a more complex component of the immune system. It is activated 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 them. The effect of prebiotic or probiotic supplementation on the adaptive immune system has not been studied extensively, but some of its components appear to be enhanced. Additional research in this area is warranted to more fully understand the effects of prebiotics and probiotics on adaptive immunity.

Disease resistance

The ability of the cultured organism to resist disease from an infectious agent is critical because it directly affects the production efficiency and profitability of the enterprise. Disease resistance is an integrated response or outcome that may be influenced by the organism's genetic makeup and by various components of the immune system.

Numerous studies have demonstrated that prebiotic and probiotic supplements may enhance the ability of various aquatic species to resist disease from bacterial, viral and protozoan pathogens (Table 1). For example, the prebiotic GroBiotic®-A has enhanced survival of hybrid striped bass exposed to *Streptococcus iniae* and *Mycobaterium marinum*, Nile tilapia (*Oreochomis niloticus*) exposed to *Aeromonas hydrophila* and *Streptococcus iniae,* and golden shiner (*Notemigonus chrysoleucus*) exposed to *Flavobacterium columnare*. Protection from other pathogens also has been reported. For example, rainbow trout (*Oncorhynchus mykiss*) fed a diet supplemented with GroBiotic®-A at 2% by weight had significantly greater survival after exposure to infectious hematopoietic necrosis virus. Similar improvements in survival were observed for red drum (*Sciaenops ocellatus*) fed GroBiotic®-A at 1% by weight before exposure to the parasitic dinoflagellate *Amyloodinium ocellatum*.

Nutrient utilization

The enhancement of certain beneficial bacteria in the GIT has been associated with improved digestion of dietary nutrients and energy by some fish species. Red drum fed diets in which equal amounts of protein were provided by fish meal and soybean meal had higher digestibility coefficients for protein, energy, and organic matter when the dairy-yeast prebiotic GroBiotic®-A, mannanoligosaccharide (MOS), or galactooligosaccharide (GOS), but not inulin, was individually added to the diet at 1% by weight (Burr et al., 2008b). The specific mechanism for increased nutrient digestibility was not determined in that study. However, the increased nutrient digestibility associated with prebiotic or probiotic supplementation may be due to the favored microbial community producing enzymes that are either lacking or occurring only at low levels in the host (reviewed by Burr et al., 2005). For example, increases in protease, lipase, amylase and cellulose enzyme activities were observed in white shrimp (*Litopenaeus vannamei*) fed freeze-dried *Rhodobacter sphaeroides* and *Bacillus* sp. probiotics (Wang, 2007).

Recent studies have shown that prebiotics may increase the absorptive area of the GIT, based on quantitative changes in histological measurements of the GIT such as intestinal fold height, enterocyte height, and microvillus height (Dimitroglou et al., 2010; Zhou et al., 2010). Such changes also could contribute to increased nutrient absorption. The increased nutrient utilization and enhanced metabolism associated with prebiotics and probiotics also may result in increased weight gain and feed efficiency. Such improvements have been most readily observed when organisms are cultured under less than optimal environmental conditions or in the presence of pathogenic organisms.

Other interactions

As new research techniques are developed to assess the endocrine and molecular effects of prebiotic and probiotic supplementation, additional insights on how these compounds influence metabolism are likely to emerge. For example, European seabass (*Dicentrarchus labrax*) larvae fed a *Lactobacillus* sp. probiotic via rotifers and *Artemia* had increased weight gain associated with increased insulin-like growth factor (IGF)-I expression based on mRNA transcription and a decrease in myostatin mRNA transcription (Carnevali et al., 2006). It also was noted in that study that the whole-body content of the stress hormone cortisol in larval seabass was reduced after 70 days of exposure to the probiotic, compared to the control group. This indicates that the probiotic reduced stress on the fish. Such a response is of considerable interest given the immunosuppressive effect of cortisol on fish. Golden shiner intentionally exposed to handling stress before disease exposure had significantly higher whole-body cortisol levels and higher mortality when fed a basal diet compared to those fed a diet supplemented with GroBiotic®-A (Lochmann et al., 2010).

Practical application of prebiotics and probiotics

The viability of probiotics must be maintained during storage and processing for them to exert their beneficial effects on the cultured species. 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 after extrusion so the probiotic organism is not exposed to excessive heat and pressure. Administering probiotics in the form of lyophilized cells or spores may be less demanding.

Feed manufacturing constraints are generally of less concern when dealing with prebiotics because they are not living organisms. Although several prebiotics have been shown to be efficacious when incorporated into extrusion-processed feeds, the potential chemical alteration of prebiotic compounds during feed manufacturing has not been studied widely.

Administration regimes for specific prebiotics and probiotics 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. Therefore, these diet additives may be administered for extended periods. However, more refined administration protocols for individual prebiotics or probiotics should be investigated to optimize their effectiveness. For example, administering probiotics or prebiotics at prescribed times before the cultured organism is exposed to a stressful event, or at particular times of the year when pathogenic organisms are most prevalent, may be the most efficient way to derive benefits from these compounds under particular culture regimes.

Other considerations

A number of probiotics and prebiotics are now commercially available and in use. Although there are added costs associated with using these products, improved production efficiency and reduced disease incidence may offset such costs.

Another possible way to use prebiotics and probiotics is to administer compounds from both groups at the same time. Such a combination is termed a synbiotic. A synbiotic is intended to improve the survival and implantation of the live microbial supplement in the GIT. While there has been limited research on the use of synbiotics in aquaculture, the many positive effects of prebiotics or probiotics may lead to the development of protocols for administering combinations of these compounds.

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USDA United States Department of Agriculture National Institute of Food and Agriculture

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