Evidence of the beneficial effects of probiotics gave rise to the concept of prebiotics, which are defined as nondigestible 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 intestinal tract, thus improving the host's intestinal balance. Information pertaining to application of prebiotics in aquaculture is extremely limited to date. Research by the authors has shown that dietary supplementation with a commercial prebiotic significantly enhanced growth and disease resistance of hybrid striped bass beyond that achieved with brewers yeast.

Dietary Supplementation of Prebiotics for Health Management of Hybrid Striped Bass *Morone chrysops x M. saxatilis*

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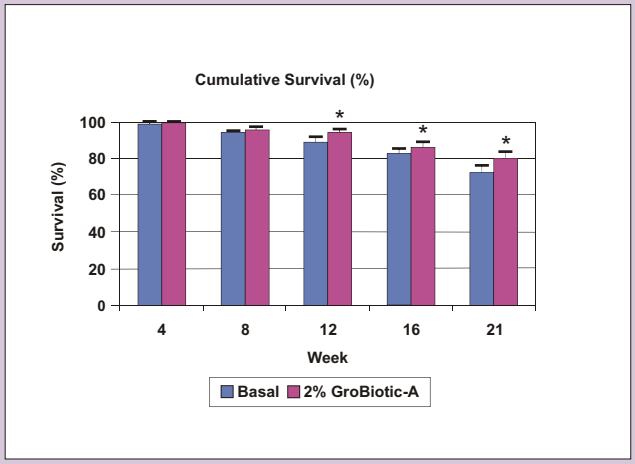
Introduction

Although the concept of functional feeds is relatively new to the aquaculture industry, it represents an emerging new paradigm to develop diets that extend beyond satisfying basic nutritional requirements of the cultured organism to improving their health and resistance to stress and disease-causing organisms. Increasing concerns about antibiotic residues in food products have resulted in more restrictive use of antibiotics to treat bacterial diseases in various animal industries (Patterson and Burkholder 2003). These alterations in drug-use policy and consumer attitudes may impact aquaculture and thus have prompted interest in developing alternative strategies for disease control. Hybrid striped bass (*Morone chrysops x M. saxatilis*) is an important farmed fish in the United States and is negatively impacted by various bacterial pathogens such as *Streptoccocus iniae* and *Mycobacterium marinum* (Plumb 1997). Disease prevention and treatment strategies such as use of vaccines and drugs are currently limited in hybrid striped bass aquaculture due to regulatory constraints or inconvenient administration protocols. Therefore, this fish has been used in our laboratory as a model for investigating the effects of various dietary factors on disease resistance as well as immunocompentence. Our research has focused on selected nutrients such as Vitamin C, Vitamin E and selenium, as well as various natural feedstuffs and synthetic compounds which may enhance fish health. This article summarizes some recent findings with one of the most beneficial diet supplements evaluated to date, a commercial prebiotic that has been shown to enhance growth of hybrid striped bass as well as combat the adverse effects of the bacterial pathogens *S. iniae* and *M. marinum*.

Defining Probiotics and Prebiotics

Probiotics were originally defined as live microbial feed supplements that alter the microbiota of the gastrointestinal (GI) tract which is recognized as playing

Mycobacteriosis generally is a chronic disease that may cause unmarketable appearance and high cumulative mortality in hybrid striped bass. Our research has shown that dietary supplementation of the commercial prebiotic Grobiotic®-A provided significantly reduced mortality from this disease. In the graph, an asterisk (*) indicates a significant (P<0.05) difference between the basal and Grobiotic®-A-supplemented diets at each specific time



important roles in the growth, digestion, immunity and disease resistance of the host organism. Based on early research with humans and terrestrial livestock, potential use of probiotics in aquaculture received heightened attention (Gatesoupe 1999, Gatlin 2002, Irianto and Austin, 2002). Although some probiotics used in aquaculture are designed to treat the aquatic environment for competitive exclusion of potential pathogens, most probiotics are delivered to hosts by dietary supplementation. Besides various strains of bacteria, other organisms such as bacteriophages, fungi and microalgae also have been incorporated in diets as probiotics.

Evidence of the beneficial effects of probiotics gave rise to the concept of prebiotics, which are defined as nondigestible 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 intestinal tract, thus improving the host's intestinal balance (Gibson and Roberfroid 1995). Examples of prebiotics include mannanoligosaccharides (White et al. 2002), lactose (Szilagyi 2002), as well as oligofructose and inulin (Teitelbaum & Walker 2002).

Information pertaining to application of prebiotics in aquaculture is extremely limited to date. The earliest studies showed that dietary fatty acids (Ringø et al. 1998) and carbohydrates (Ringø and Olsen 1999) could alter bacterial flora in the GI tract of Arctic charr Salvelinus alpinus. In a subsequent study (Olsen et al. 2001), it was observed that a diet supplemented with 15% inulin adversely affected the enterocytes of this fish.

Evaluation of the prebiotic Grobiotic®-A with hybrid striped bass

To explore potential use of a specialized prebiotic preparation in aquaculture, our laboratory evaluated the commercial product Grobiotic®-A (International Ingredient Corp., St. Louis, MO, USA) in three separate feeding trials with hybrid striped bass. This product is a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products containing 35.2% crude protein, 1.7% crude lipid and ~53% simple and complex carbohydrates including oligosaccharides. A similar product has been shown to influence the intestinal microflora of chickens as well as enhance their growth (Halpin, K. M., International Ingredient Corp., personal communication). In each of our trials, a basal diet was formulated to contain 40% crude protein from menhaden fish meal, 10% lipid and 14.6 kJ digestible energy/g. Experimental diets were supplemented with Grobiotic®-A at either 1% or 2% of dry weight and maintained isonitrogenous and isoenergetic by adjusting the amount of fish meal and dextrin. Because partially autolyzed brewers yeast is a constituent of Grobiotic®-A, we also included diets supplemented with brewers yeast (1 and 2% of diet) while the basal diet (nonsupplemented) was the negative control. After a 7-week period in the first feeding trial, significantly enhanced weight gain (% of initial weight) and feed efficiency (g weight gain/g feed) were observed in juvenile fish (initial weight of 7 g/fish) fed diets supplemented with 1 or 2% Grobiotic®-A compared to those fed the basal diet (Li & Gatlin 2004a). Neither 1% nor 2% brewers yeast provided a similar influence on weight gain or feed efficiency compared to the prebiotic-supplemented diets. Survival during feeding trial 1 was high and no significant differences were observed among treatments. In the second feeding trial, survival of juvenile fish (initial weight of 19.7 g/fish) fed diets containing Grobiotic®-A or brewers yeast for 4 weeks and then exposed by immersion to a standardized dose of Streptococcus iniae was significantly ($P< 0.01$) higher than fish fed the basal diet. Extracellular superoxide anion production of head kidney macrophages from fish fed Grobiotic®-A or brewers yeast also was significantly higher than that of fish fed the basal diet. We failed to observe significantly different effects of dietary Grobiotic®-A and brewers yeast on performance of juvenile hybrid striped bass in this short-term trial.

To further explore possible age/size-related responses, a 21-week feeding trial was conducted to evaluate Grobiotic®-A in the diet of sub-adult hybrid striped bass (initial weight of 65-118 g/fish) exposed to a chronic mycobacterial infection caused by *Mycobacterium marinum* (Li & Gatlin 2004b). Enhanced growth performance was generally observed in fish fed diets supplemented with Grobiotic®-A or brewers yeast compared to fish fed the basal diet throughout the feeding trial with significantly ($P<0.05$) enhanced weight gain observed after 12 weeks of feeding. The in situ mycobacterial challenge employed in this experiment resulted in overall cumulative mortality of approximately 25%. Fish fed 2% Grobiotic®-A had a significantly ($P<0.05$) enhanced survival (80%) compared to the other treatments (72-73%) at the end of 21 weeks.

Based on knowledge acquired from human and terrestrial animals, prebiotics are usually most effective against enteric diseases. It is known that ingestion of feed is a port of entry for mycobacteria in some fish species such as snakehead *Channa striatus* (Chinabut et al. 1990). This could possibly be a factor contributing to the positive response associated with the Grobiotic®-A supplement. Some researchers also have suggested that the interaction between intestinal microflora and enterocytes may trigger release of biologically-active substances and influence host immunity (reviewed by Patterson & Burkholder 2003). Specific mechanisms by which Grobiotic®-A has positively influenced disease resistance of hybrid striped bass are currently being explored.

Conclusions

Our research has shown that dietary supplementation of the prebiotic Grobiotic®-A significantly enhanced growth and disease resistance of hybrid striped bass beyond that achieved with brewers yeast. Similar observations have been made with other fish species including red drum (*Sciaenops ocellatus*) and tilapia (*Oreochromis* sp.) in our recent experiments. The consistent benefits observed with this prebiotic indicate it may be very helpful to the aquaculture industry. Future studies in our laboratory will include targeting the dynamics of intestinal microbiology in response to prebiotics, screening for beneficial probiotics and developing novel prebiotics and symbiotics (a probiotic together with a prebiotic) for health management of hybrid striped bass and other fish species. ■

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