

# Strategies of Dietary Supplementation for Health Management of Hybrid Striped Bass *Morone Chrysops* X *M. Saxatilis*: A Review of Research at Texas A&M University

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## Abstract

A series of experiments has been conducted with hybrid striped bass in our laboratory to evaluate several different dietary additives that may influence immune response and disease resistance. These include glucans, levamisole, partially autolyzed brewers yeast, oligonucleotides and a yeast-based prebiotic mixture. Some of these supplements such as brewers yeast at 2% of diet significantly enhanced blood neutrophil oxidative radical and extracellular superoxide anion production of head kidney macrophages as well as enhanced resistance to *S. iniae*. Additional experiments confirmed this observation in sub-adult hybrid striped bass and showed some protection against *M. marinum* after an *in situ* infection. The prebiotic Grobiotics<sup>®</sup>-A, a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products, provided a surprisingly enhanced growth and feed efficiency hybrid striped bass as well as immunostimulation and enhanced resistance to *S. inae* and *M. marinum* when included at 2% of diet. Dietary nucleotides also have been demonstrated to enhance neutrophil function and resistance of hybrid striped bass to *S. iniae* infection, although the mechanisms behind these phenomena are not well defined.

Although the concept of functional feeds is novel to the aquaculture industry, it represents an emerging new paradigm to develop diets that extend beyond satisfying basic nutritional requirements of the cultured organism. Our observation with hybrid striped bass have shown that research in this area is worthy to pursue further and may be very helpful for the industry.

Key words: immunonutrient, immunostimulant, health, hybrid striped bass, *Morone*

Running title: Nutrition and health of hybrid striped bass

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## Introduction

Hybrid striped bass production is considered to be the fastest growing segment of the U.S. aquaculture industry over the past decade and is poised to become a global seafood delicacy in the twenty-first century (Harrell & Webster 1997; Kohler 2000). However, this industry is negatively impacted by various pathogenic organisms (Plumb 1997). In recent years, there have been growing concerns about the adverse effects of the bacterium *Streptococcus iniae* in the aquaculture of many economically important marine and freshwater fish species (reviewed by Li & Gatlin 2003). This bacterium may cause heavy losses from mortality,

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reduced growth as well as unmarketable appearance in various fish species. Unfortunately, hybrid striped bass is one of the most susceptible fish to *Streptococcus iniae* infection, and this bacterium also is associated with acute cellulitis in humans. All reported occurrences of the human disease were associated with puncture wounds or abrasions and handling of infected fish or contaminated water (Greenlees, Machado, Bell & Sundlof 1998).

Besides *Streptococcus iniae*, the prevalence of mycobacteria such as *Mycobacterium marinum* wild striped bass and cultured hybrid striped bass has attracted increased attention (Overton, Margraf, Weedon, Pieper & May 2003). Although mycobacteriosis is generally chronic, this disease may cause severe infection and high cumulative mortality in closed, recirculating systems (Plumb 1997). In addition, this bacterium is reported to be capable of surviving under many adverse environmental conditions, including low temperature, and may cause infection on the extremities of humans (Plumb 1997; Mediel, Rodriguez, Codina & Martin-Casabona 2003). A recent report of the presence of mycobacteria in frozen seafood also raised concern about the safety of such seafood for human consumers (Mediel et al., 2003). Currently, effective treatment of this disease is very limited as reported for other fish species (Colorni, Avtalion, Knibb, Berger, Colorni & Timan 1998). Therefore, effective preventive strategies are not only needed to limit economical loss in aquaculture, but also to protect the health of aquaculturists and fish processing workers.

In aquaculture, traditional methods for treating infective pathogens include a limited number of government-approved antibiotics and chemotherapeutics. However, their disadvantages such as marginal effectiveness and high cost are obvious (Sealey & Gatlin, 2001). These treatments also may cause the accumulation of chemicals in the environment and/or fish, thus posing potential threats to consumers and the environment. Although progress has been made in development of improved drugs (Darwish & Ismaiel 2003) and vaccines (Buchanan, Stannard, Westerman, Ostland, Van Olst, Carlberg & Nizet 2003) against *Streptococcus iniae*, these strategies are still not readily implemented due to regulatory limitations or inconvenient administration protocols. In the United States, there is no therapeutic approved by the U.S. Food and Drug Administration specifically for

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hybrid striped bass. In addition, increasing concerns of antibiotic use have resulted in a ban on subtherapeutic antibiotic usage by various animal industries in Europe and the potential for a ban in the United States and other countries (Patterson & Burkholder 2003). These alterations in policy may impact aquaculture and thus have prompted additional interest in developing alternative strategies for disease control such as immunostimulation (Gannam & Schrock 2001).

Research on the subject of nutritional modulation, especially evaluation of natural extracts or synthetic compounds which may enhance the immune responses and disease resistance of hybrid striped bass is of special importance and urgency. Therefore, this fish has been used in our laboratory as a model for investigating the interactions between diet and disease resistance as well as providing dietary strategies for the hybrid striped bass industry.

Beginning in 2000, our laboratory has conducted a series of experiment to evaluate various nutrients and other dietary factors as well as nutritional strategies to enhance immune responses and disease resistance of hybrid striped bass. Earlier studies involved micronutrients such as vitamin C, vitamin E (Sealey & Gatlin 2002a, 2002b), and selenium (Jaramillo and Gatlin 2004), while more recently various dietary supplements have been evaluated including yeast  $\beta$ -glucans, partially autolyzed brewers yeast, oligonucleotides, levamisole as well as the prebiotic Grobiotic<sup>®</sup>-A (a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation A summary of the results from these studies of various diet additives, and explanations/hypotheses generated by this research are summarized in the following sections.

## **Yeast glucans**

The immunomodulatory effects of  $\beta$ -glucans have been observed in various aquatic animals (reviewed by Gatlin 2002; Misra, Das, Pradhan, Pattnaik, Sethi & Mukherjee 2004) as well as many terrestrial species (e.g., Guo, Ali & Qureshi 2003; Davis, Murphy, Brown, Carmichael, Ghaffar & Mayer 2004). Extracted from brewers yeast, a by-product of the

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brewing industry, yeast glucans have become affordable for the aquaculture industry. In addition, discoveries of glucan receptors such as Dectin-1 and Toll-like receptors on leukocyte surfaces of vertebrates have shed some light on mechanisms of immune responses induced by  $\beta$ -glucans (reviewed by Brown & Gordon 2003). Potential use of dietary glucans to reduce risks from infectious diseases in hybrid striped bass has been investigated. Jaramillo and Gatlin (2004) did not observe any positive effects of supplementing the diet with  $\beta$ -glucans from barley. However, more recently, Li & Gatlin (unpublished data) supplemented menhaden fish meal-based diets with three incremental levels (1, 2 and 4% of diet) of MacroGuard<sup>®</sup>, a source of highly purified  $\beta$ -1, 3- glucans from brewers yeast *Saccharomyces cerevisiae* (KS Biotec Inc., Tromsø, Norway) and fed the diets to juvenile hybrid striped bass. After 4 weeks of feeding, some immune responses were determined and a bath challenge with *S. iniae* was conducted. Fish fed yeast glucan-supplemented diets have increased blood neutrophil oxidative radical production and those fish fed 0.1% MacroGuard<sup>®</sup> had a remarkable and significant reduction in mortality (10%), compared with fish fed the basal diet (46.7%). The diets supplemented with 0.05 and 0.2% MacroGuard<sup>®</sup> showed some protection based on limited mortality (25%); however, survival of these fish was not significantly different from those fed basal diet. It is concluded that dietary supplementation of yeast glucans could reduce the risk and mortality caused by *S. iniae* infection. However, more research is needed to clarify the possible effects of dose and administration length to optimize use of yeast glucans for hybrid striped bass culture. Although there currently appears to be no consistent conclusion about the efficacy of long-term administration of yeast glucans, several research groups have generated both *in vitro* and *in vivo* evidence that inappropriate use of  $\beta$ -glucans may suppress immunity at the cellular and organismal levels (Novoa, Figueras, Ashton & Secombes 1996; Jeney, Galeotti, Volpatti, Jeney & Anderson 1998; Castro, Couso, Obach & Lamas 1999; Couso, Castro, Margarinos, Obach & Lamas 2003).

## Brewers yeast

Yeast by-products from the brewing industry are natural diet additives that have been shown to positively influence non-specific immune responses of some fish species such as rainbow trout *Oncorhynchus mykiss* (Walbaum) and gilthead sea bream *Sparus auratus* (L.) (Siwicki, Anderson & Rumsey 1994; Ortuño, Cuesta, Rodríguez, Esteban & Meseguer 2002). Our laboratory has conducted five separate feeding trials to evaluate supplementation (1, 2, or 4%) of partially autolyzed brewers yeast (International Ingredient Corp., St. Louis, MO, USA) in the diet of juvenile and sub-adult hybrid striped bass. Based on our results, low dose supplementation of brewers yeast generally had marginally positive effects on growth under normal conditions. Although sometimes performance enhancement was rather noticeable (Li & Gatlin 2003), it was not consistent when comparing all feeding trials. Most recently, 2% brewers yeast significantly enhanced growth but not survival of sub-adult hybrid striped bass chronically infected with *M. marinum*, (Li & Gatlin, 2004b). Dietary supplementation with brewers yeast enhanced survival after bath challenge with *S. iniae* after 4 or 8 week of feeding (Li & Gatlin 2003; 2004), as well as increased respiratory burst of blood neutrophils and head kidney macrophages after 4 weeks (Li & Gatlin 2004a) or 16 weeks of feeding (Li & Gatlin 2003). These observations have led us to believe that dietary supplementation of brewers yeast could be administered over a rather extended period without causing obvious immunosuppression (Li & Gatlin 2003). However, the use of respiratory burst of neutrophils or macrophage function as effective biomarkers for disease resistance is uncertain based on some very recent research which showed that respiratory burst of invertebrate phagocytes was negatively related to survival after *Vibrio alginolyticus* exposure (Tseng & Chen 2004; Yeh, Liu & Chen 2004). The long-term administration of brewer yeast needs to be re-evaluated based on information from challenges with various pathogens.

## Oligonucleotides

Oligonucleotides traditionally have not been considered to be essential nutrients because they are synthesized by the body and signs of deficiency do not result when they are excluded from the diet. Therefore, the nutritional value of nucleotides has been debated for many years. However, this opinion has been punctured by successive research publications which suggest that nucleotide requirements increase during injury and/or wound repair, and deficiency may impair liver, heart, intestine and immune functions (Grimble & Westwood 2000). Although initial efforts in exploration of nucleotide nutrition in fishes could be traced to the early of 1990s (Ramadan & Atef 1991; Ramadan, Afifi, Moustafa & Samy 1994; Adamek, Hamackova, Kouril, Vachta & Stibranyiova 1996), world-wide heightened attention was aroused by the report of Burrells, William & Forno (2001). Surprisingly, research pertaining to dietary nucleotide supplementation in fishes showed rather consistent and encouraging results, suggesting strong potential for supplementation of nucleotides in formulated aquafeeds to influence immune responses and enhance disease resistance (Burrells et al. 2001; Burrells, William, Southage & Wadsworth 2001; Sakai, Taniguchi, Mamoto, Ogawa & Tabata 2001; Leonardi, Sandino & Klempau 2003; Low, Wadsworth, Burrells & Secombes 2003) of various fish species. However, the molecular mechanism(s) of physiological and immunological responses induced by dietary nucleotides remains unknown. It is currently known that tissues such as the intestinal mucosa, bone marrow hematopoietic cells, lymphocytes and the brain have limited capacity for *de novo* nucleotide synthesis and depend on their supply from the salvage pathway. Therefore, the endogenous supply of nucleotides may not be adequate for optimal functioning, especially of the immune system, under stressful conditions such as sepsis and trauma, and thus a dietary supply may be of particular significance (Yamauchi, Hales, Robinson, Niehoff, Ramesh, Pellis & Kulkarni (2002).

Three feeding trials have been conducted to evaluate potential immunomodulatory effects of dietary oligonucleotides on hybrid striped bass (Li, Lewis & Gatlin 2004a). Oligonucleotides from Ascogen P<sup>®</sup> (Canadian Bio-Systems Inc., Calgary, Alberta, Canada)

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were added to the basal formulation at the manufacturer's recommended rate of 0.5% and fed to hybrid striped bass for 8 (trial 1), 7 (trial 2) and 16 (trial 3) weeks. Growth was not influenced by dietary supplementation of oligonucleotides; however, significantly ( $p=0.017-0.091$ ) enhanced survival after exposure to *S. iniae* was generally observed in each controlled challenge after trial 1. In trial 2, fish fed the nucleotide-supplemented diet tended to have a higher antibody response based on microtitration agglutination and slide agglutination; however, the difference was not statistically significant because of high variation among individual fish. Neutrophil oxidative radical production of fish fed the nucleotide-supplemented diet was significantly higher than that of fish fed the basal diet. In the third trial, intracellular and extracellular superoxide anion production of head kidney macrophages, neutrophil oxidative radical production and serum lysozyme were not significantly affected by long-term administration of dietary nucleotides when compared to fish fed the basal diet. Leonardi et al. (2003) also observed that fish fed nucleotide supplemented diet showed a mitogenic response of LPS-stimulated cells after 60 days of feeding, but not 120 days of feeding. Thus, information concerning the efficacy of long-term nucleotide administration is currently limited and further research is warranted.

### **Levamisole**

Levamisole, an antihelminthic drug commonly administered to terrestrial livestock, has been shown with several aquatic species to be a potent immunostimulant by modulating T-cell function (Renoux 1980), cytotoxic activity of leukocytes (Cuesta, Esteban & Meseguer 2002), phagocytosis (Mulero, Esteban, Munoz & Meseguer 1998), respiratory burst (Mulero et al. 1998; Li and Gatlin unpublished data) and macrophage-activating factor (Mulero et al. 1998). It also has been reported to enhance resistance to amoebic gill disease of Atlantic salmon *Salmo salar* (L.) (Findlay, Zilberg & Munday 2000; Munday & Zilberg 2003) and swimbladder nematode infection of eel *Anguilla anguilla* (Geets, Liewes & Ollevier 1992) as well as improve growth performance when administered orally (Mulero et al. 1998) or by water bath (Siwicki & Korwin-Kossakowski 1988). Because of its low cost and marginal residue in fish, levamisole has been tested for its immunomodulatory

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effects on hybrid striped bass and for enhancing resistance to pathogenic bacteria including *S. iniae* and *Aeromonas hydrophila*. The same menhaden fish meal-based basal diet as previously mentioned (containing 40% protein, 10% lipid and an estimated digestible energy level of 3.5 kcal/g) was supplemented with four levels (100, 250, 500 and 1000 mg/kg) of levamisole in place of cellulose. Although intracellular superoxide anion production of head kidney macrophages of hybrid striped bass fed 250 mg levamisole/kg diet was significantly higher than that of the other four groups after 4 weeks of feeding, this beneficial influence was not correlated with resistance to *S. iniae* infection. No significant difference in survival after *S. iniae* challenge (trial 1) or *A. hydrophila* challenge (trial 2) was observed after 4-weeks (trial 1) or 3 weeks (trial 2) of feeding the experimental diets. However, supplementation of a low dose (0.1 g/kg<sup>-1</sup>diet) of levamisole significantly enhanced weight gain and feed efficiency (P<0.01) after 3 weeks of feeding. In contrast, a high dose (1 g/kg<sup>-1</sup> diet or approximately 56 mg/kg fish weight per day) of levamisole caused chronic toxicity and retarded growth of hybrid striped bass (Li, Wang & Gatlin 2004b). In addition, a series of *in vitro* manipulations were conducted with phagocytic cells isolated from the head kidney (HK) of hybrid striped bass. The HK phagocytes were cultured in media containing 1, 10, 100, 1000 µg levamisole/ml. Extracellular and intracellular reactive oxygen intermediates were determined after 24, 48 and 72 h of incubation. No differences in reactive oxygen intermediates were observed among the treatments; however, 1000 µg levamisole/ml significantly (p<0.0001) suppressed the generation of reactive oxygen. Also, 100 µg levamisole/ml suppressed HK cell functions after 48-h and 72-h incubations (Li et al. 2004b).

## Prebiotics

Probiotics, defined as live microbes administered as dietary supplements to improve intestinal microbial balance, have received some attention in aquaculture (Gatesoupe 1999; Irianto & Austin 2002). Evidence of the beneficial effects of probiotics gave rise to the concept of prebiotics (Gibson & Roberfroid, 1995; Teitelbaum & Walker, 2002), which were defined by Gibson & Roberfroid (1995) as “a nondigestible food ingredient which

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beneficially affects 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". Examples of prebiotics include mannan oligosaccharides (White, Newman, Cromwell & Lindemann 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. Olsen, Myklebust, Kryvi, Mayhew & Ringø (2001) observed that a diet supplemented with 15% inulin caused harmful effects to Arctic charr *Salvelinus alpinus* (L.). However, their previous studies (Ringø, Bendiksen, Gausen, Sundsfjord & Olsen 1998; Ringø & Olsen, 1999) showed that dietary fatty acids and carbohydrates altered the bacterial flora of the gastrointestinal tract of fish.

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). 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 chicken as well as enhance their growth (Halpin, K. M., International Ingredient Corp. personally communication).. Because brewers yeast is a constituent of "Grobiotic®-A", we also included a diet supplemented with brewers yeast as a positive control while the basal diet (nonsupplemented) was a negative control in three separate feeding trials to distinguish effects of this prebiotic mixture to brewers yeast. After a 7-week feeding period in the first feeding trial, significantly enhanced weight gain (% increase in biomass accumulation) and feed efficiency (g weight gain/g feed) were observed in fish fed diets supplemented with 1 and 2% Grobiotic®-A compared to those fed the basal diet. Neither 1% nor 2% brewers yeast provided a similar influence on growth, compared to the prebiotic-supplemented diet. Survival during feeding trial 1 was high and no significant differences were observed among treatments (Li & Gatlin 2004a). In the second feeding trial, survival of fish fed diets containing Grobiotic®-A or brewers yeast was significantly ( $P < 0.01$ ) higher than fish fed

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the basal diet after 4 weeks. Extracellular superoxide anion production of head kidney cells from fish fed Grobiotic<sup>®</sup>-A or brewers yeast also was significantly higher than those of fish fed the basal diet. We failed to observe significantly different effects of dietary Grobiotic<sup>®</sup>-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<sup>®</sup>-A in the diet of hybrid striped bass exposed to chronic mycobacterial infection caused by *Mycobacterium marinum*, as compared to partially autolyzed brewers yeast (Li & Gatlin 2004b). Enhanced growth performance was generally observed in fish fed diets supplemented with Grobiotic<sup>®</sup>-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<sup>®</sup>-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 also is a port of entry for mycobacteria in some fish species including snakehead *Channa striatus* (Fowler)(Chinabut, Limsuwan & Chanratchakool 1990). This could possibly be a factor contributing to the positive response associated with the Grobiotic<sup>®</sup>-A supplement. Some researchers suggested that interaction between intestinal microflora and enterocytes may trigger release of biologically-active substances and influence host immunity (reviewed by Patterson & Burkholder 2003). Although further evidence from intestinal microbiology is still needed to confirm proper use of the term “prebiotic”, the encouraging results from our studies provide an interesting foundation for future research.

## Future Research

Although the concept of functional feeds is novel to the aquaculture industry, it represents an emerging new paradigm to develop diets that extend beyond satisfying basic nutritional requirements of the cultured organism. Our observation with hybrid striped bass have

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shown that research in this area is worthy to be pursued further and may be very helpful to the aquaculture industry. Future research in this laboratory will include evaluating:

Refine dose and administration duration of immunostimulants to optimize their effectiveness in hybrid striped bass.

Explore other immunostimulants such as chitin, lactoferrin, peptidoglycan, liposaccharides, sulfated polysaccharide, 3, 3', 5- triiodo-L-thyronine,  $\beta$ -hydroxy- $\beta$ -methylbutyrate, as well as certain bacterins and fungal products.

Determine intestinal microfloral changes after dietary treatment with prebiotics and beneficial microbes (probiotics), as well as develop prebiotics and symbiotics (a probiotic together with a prebiotic) dietary intervention.

Utilize molecular methods such as microarray analysis to precisely measure influences of immunostimulants on expression of immunogenes and screen for effective dietary immunostimulants.

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