North American Journal of Aquaculture 69:305–308, 2007

DOI: 10.1577/A06-078.1

[Article]

Mortality Rates in Golden Shiners Fed High-Fat Diets with or without a Dairy-Yeast Prebiotic before Challenge with *Flavobacterium columnare*

TODD D. SINK,* REBECCA T. LOCHMANN, ANDREW E. GOODWIN, AND EMILY MARECAUX

University of Arkansas at Pine Bluff, Department of Aquaculture and Fisheries, 1200 North University Drive, Mail Slot 4912, Pine Bluff, Arkansas 71601, USA

Abstract.—Columnaris, caused by *Flavobacterium columnare*, is a major bacterial disease of the golden shiner *Notemigonus crysoleucas*, especially when fish become stressed during handling and transport. Feed additives, such as a dairy-yeast prebiotic, can decrease disease susceptibility in some fish species. Previous studies have indicated that diets with higher fat concentrations have improved the growth and survival of golden shiners. We conducted a study to determine whether a high-fat diet alone or supplemented with a dairy-yeast prebiotic could decrease mortality rates of golden shiners subjected to columnaris challenge. Golden shiners were assigned one of three diets with four replicate aquaria per diet and fed to apparent satiation twice daily for 16 weeks before the challenge. Diets (30.3 ± 0.31% crude protein; mean ± SE) were similar to a commercial formula and contained (1) 4% poultry fat (control), (2) 10% poultry fat, or (3) 10% poultry fat and 2% dairy-yeast prebiotic. After the 16-week feeding period, 15 golden shiners (2.2 ± 0.03 g) from each aquarium were stocked into different aquaria (experimental replicates were maintained) and exposed to 20 mL of columnaris bacteria in Sheih broth (optical density, 0.395 Å; at 560 nm; Sheih broth blank) for 18 h. Kidney cultures taken from moribund fish were streaked on Sheih agar to confirm the presence of columnaris and an active infection. Mortality in the 4% poultry fat (41.7 ± 12.9%) and 10% poultry fat (40.0 ± 6.1%) diets was high and not significantly different. Mortality for the dairy-yeast prebiotic (6.7 ± 2.7%) diet was much lower and significantly different from that for the 4% and 10% poultry fat diets. Thus, the dairy-yeast prebiotic effectively reduced mortality rates in golden shiners exposed to columnaris, but a high-fat diet alone provided no protection relative to the lower-lipid control diet.

Arkansas is the leading baitfish producer in the United States, and the golden shiner *Notemigonus crysoleucas* accounts for the majority of production (Table 1 in NASS 2006). Columnaris, caused by *Flavobacterium columnare*, is a major bacterial disease that accounts for heavy losses in golden shiners, especially when the fish become stressed during handling and transport. Investigation of new feed additives that could help maintain golden shiner health and reduce mortality due to columnaris is warranted.

The use of high-fat diets for golden shiners is a possible way to decrease columnaris outbreaks. Previous studies using higher-fat diets have presented positive health aspects for golden shiners (Lochmann and Phillips 2001). Commercial feeds currently in use for golden shiners contain 3–4% lipid. Shiners fed 4% and 13% fat diets had similar growth, but survival of the shiners receiving the high-fat diet was significantly greater than that of those receiving the standard 4% fat commercial diet (Lochmann and Phillips 2001). Fish health is known to be impacted by dietary lipid content (Blazer 1992; Fracalossi and Lovell 1994).

* Corresponding author: tsink@uaex.edu

Received October 18, 2006; accepted February 6, 2007
Published online July 26, 2007

Recent evidence suggests that prebiotic feed additives can decrease disease susceptibility in some fish species (Li and Gatlin 2004, 2005). Prebiotics are ingestible carbohydrates that often contain high concentrations of oligosaccharides (Vazquez et al. 2006). Prebiotics are used to stimulate the growth and activity of beneficial intestinal bacteria and may also stimulate synthesis of vitamin B_6 (Aylward and Bofinger 2006). Prophylactic use of orally administered antibiotics to maintain fish health is declining owing to concerns about producing antibiotic-resistant bacteria and the potential adverse effects on beneficial intestinal bacteria. Prebiotics have the potential to reduce or replace antibiotic use in fish health maintenance (Li and Gatlin 2005).

Li and Gatlin (2004) reported increased survival in hybrid striped bass (white bass *Morone chrysops* × striped bass *M. saxatilis*) fed dairy-yeast prebiotic diets (73.3%) compared with that in fish fed basal diets (53.3%) following a challenge with *Streptococcus iniae*. Hybrid striped bass fed diets with dairy-yeast prebiotics also experienced enhanced survival (80%) compared with those fed diets with brewer's yeast or no supplements (72–73%) following exposure to *Mycobacterium marinum* (Li and Gatlin 2005). Rainbow trout *Oncorhynchus mykiss* fed diets containing brew-

TABLE 1.—Composition of diets (percent as fed) containing different concentrations of poultry fat (PF) and dairy-yeast prebiotic (DYP) fed to golden shiner in tanks for 16 weeks before challenge with *Flavobacterium columnare*.

Ingredient	Diet		
	4% PF	10% PF	10% PF + DYP
Menhaden Select fish meal	5.0	5.0	5.0
Poultry by-product meal	10.0	10.0	10.0
Soybean meal	43.0	43.0	43.0
Cottonseed meal	10.0	10.0	10.0
Corn	13.0	7.0	5.0
Wheat bran	13.0	13.0	13.0
Vitamin mix[a]	1.0	1.0	1.0
Mineral mix[a]	1.0	1.0	1.0
Poultry fat	4.0	10.0	10.0
GroBiotic-A[b]	0.0	0.0	2.0

[a] Same as Moon and Gatlin (1991).
[b] Donated by International Feed Ingredient Corp. (St. Louis, Missouri).

er's yeast or a dairy-yeast prebiotic had three times greater survival than those fed a control diet (61.5, 63.5, and 22% survival, respectively; W. M. Sealey, Aquaculture Research Institute, University of Idaho, personal communication). Studies using diets containing dairy-yeast prebiotics have also produced promising results in tilapia *Oreochromis* spp. and red drum *Sciaenops ocellatus* (D. M. Gatlin III, Texas A&M University, personal communication). These positive results with a variety of pathogens indicate that dairy-yeast prebiotics are potentially useful for increasing survival in other fish species exposed to pathogenic bacteria.

Therefore, we conducted a study to determine whether dairy-yeast prebiotics in high-fat diets could be used to decrease mortality rates of golden shiners subjected to columnaris challenges. Diets with high fat content (10%) ± dairy-yeast prebiotic were tested against a control diet (4% fat). These high-fat diets were chosen owing to the improved growth and survival of baitfish species that received high-fat diets in previous studies (Lochmann and Phillips 2001).

Methods

Fish.—Golden shiners ($N = 20{,}000$) were obtained from Harry Saul Minnow Farm, Inc., Prairie County, Arkansas. Fish were free of clinical signs of disease before the feeding trial and the bacterial challenge. Groups of 25 golden shiners with initial mean individual weights of 1.06 ± 0.008 g (mean ± SE) were stocked into each of four replicate 110-L tanks per diet and fed one of the experimental diets to apparent satiation twice daily for 16 weeks. Twenty-five golden shiners were then restocked into different 110-L tanks for the disease challenge (average individual weights, 2.2 ± 0.03 g), where they continued to receive their respective diets.

Diets.—The composition of the three diets (30.3 ± 0.31% crude protein) is shown in Table 1. The formula for the diet with 4% poultry fat (control) was similar to that used in commercial production of golden shiners. Diets 2 and 3 contained 10% poultry fat, and diet 3 also contained 2% GroBiotic-A, a dairy-yeast prebiotic that contains yeast and dairy products as well as dried fermentation products high in oligosaccharides (IIC 2006). Diets 2 and 3 contained more available energy than the control diet because they had higher fat content. Bulk diet ingredients were ground to obtain meal of appropriate particle size (<1 mm) before formulation. To prevent rancidity, dietary lipids were supplemented with 125 mg of ethoxyquin/kg of diet before they were incorporated into the diets. The diets were mixed in a Blend Master laboratory blender (Model B, Patterson-Kelley, East Stroudsburg, Pennsylvania) for 10 min, then combined with 400 mL of distilled water/kg of diet in a Berkel EF20 industrial mixer (Crypto Peerless Ltd., Birmingham, UK), pelleted (3-mm diameter) with a Torrey M-32 commercial grinder (Torrey, San Nicolas, Mexico), fan dried at room temperature (24°C), and kept frozen (−4°C) until use.

Experimental system.—The experimental system consisted of twelve 110-L glass aquaria with external standpipes maintaining 85 L of water in each tank. This was operated as a flow-through system with a flow rate of 1.1 L/min of dechlorinated (carbon filtration) municipal water. An air stone supplied by a regenerative blower was supplied to each tank to maintain supplemental aeration.

Flavobacterium columnare.—A virulent strain of *F. columnare* PB02–41 (Thomas-Jinu and Goodwin 2004) was prepared by inoculating 15 mL of Sheih broth with a frozen isolate. Growth was sufficient after 72 h at room temperature on a DS-500 orbital shaker (VWR Scientific Products, West Chester, Pennsylvania) to transfer the bacteria to three flasks with 250 mL each of Sheih broth. After 24 h on the DS-500 orbital shaker at room temperature, the PB02–41 isolate reached an optical density of 0.395 Å at 560 nm (Sheih broth blank) and was ready for use in the disease challenge. Bacterial concentration and exposure times for use in the challenge were previously determined from three LD-30 trials using golden shiners fed the control diet. Columnaris was then streaked onto Sheih agar plates containing a 1, 2, or 4% solution of GroBiotic-A to determine whether it had any antimicrobial properties.

Disease challenge.—Fifteen golden shiners (2.2 ± 0.03 g) from each dietary treatment were stocked into each of four flow-through aquaria per diet. The fish continued to receive their respective diets while

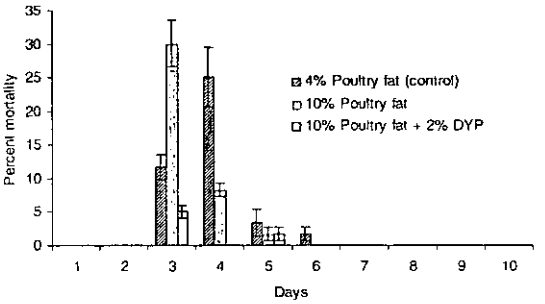

FIGURE 1.—Mean (± SE) daily mortality rates (error bars) of golden shiners fed three diets to determine whether dairy-yeast prebiotics (DYP) administered with high-fat diets decrease the mortality of golden shiners subjected to a *Flavobacterium columnare* challenge. Time is expressed as days after exposure to columnaris.

acclimating to the aquaria for 8 d; then water flow to the tanks was shut off but aeration was maintained. Twenty milliliters of *F. columnare* in Sheih broth was added to each aquarium for an 18-h exposure. After 18 h, water flow was restored and the columnaris was allowed to flush from the system. Mortality was then monitored and recorded twice daily for 10 d.

Statistical analysis.—The percent mortality of golden shiners in each treatment was analyzed using a one-way analysis of variance (ANOVA) with a Tukey's post hoc test to determine treatment differences (SPSS 2000). Data were arcsine transformed to meet the normality assumptions of the ANOVA due to a data range of greater than 40%. Differences were considered significant at $P < 0.05$.

Results

Golden shiner mortalities (Figure 1) began to occur abruptly in all treatments on day 3 and tapered to an end on day 7 (Table 2). Kidney cultures taken from moribund fish by means of a sterile technique were streaked onto Sheih agar plates to confirm the presence of columnaris and the occurrence of an active infection. Sheih agar is somewhat selective for columnaris, and the fish pathology laboratory at the University of Arkansas–Pine Bluff positively identified columnaris visually. All plates were positive for columnaris. Fish mortalities in the 4% poultry fat (41.7 ± 12.9%) and 10% poultry fat (40.0 ± 6.1%) diets were not significantly different ($P > 0.05$) from each other and higher than mortalities for the prebiotic diet. Mortality for fish fed the dairy-yeast prebiotic (6.7 ± 2.7%) diet was much lower and significantly different ($P < 0.05$) from that for fish fed the 4% or 10% poultry-fat diets.

Discussion

Growth of the golden shiners in this study was poor, as they only doubled their body weight in 16 weeks (from 1.06 to 2.2 g). Golden shiners in production ponds utilize natural foods for greater than 40% of their nutrition, even when prepared diets are used (Lochmann and Phillips 1996). Growth of golden shiners is much more rapid in production ponds (Lochmann et al. 2004), partially due to the presence of natural foods. Growth of golden shiners in indoor systems is slow (Lochmann et al. 2001) because prepared diets are the only source of dietary intake (Lochmann and Phillips 1996).

In this study, the dairy-yeast prebiotic reduced mortality rates in golden shiners fed a high-fat diet followed by exposure to columnaris. However, there was no beneficial effect of the higher fat concentration alone, as the diet with 10% fat without the dairy-yeast prebiotic did not reduce mortality relative to the control diet with 4% fat. We did not include a 4%-fat diet with the dairy-yeast prebiotic in this study owing to the

TABLE 2.—Daily and cumulative mortalities of golden shiners fed three diets to determine whether high-fat diets with or without a dairy-yeast prebiotic decrease the mortality of golden shiners subjected to a *Flavobacterium columnare* challenge.

Treatment (% poultry fat, % prebiotic)	Tank	Day							Total individuals/ treatment	Percent total	Total individuals per diet
		1	2	3	4	5	6	7			
4 (control)	23	0	0	1	3	0	0	0	4	26.7	25
4 (control)	28	0	0	1	2	0	1	0	4	26.7	
4 (control)	19	0	0	3	7	2	0	0	12	80.0	
4 (control)	26	0	0	2	3	0	0	0	5	33.3	
10	24	0	0	3	1	0	0	0	4	26.7	24
10	22	0	0	6	1	0	0	0	7	46.7	
10	17	0	0	6	1	1	0	0	8	53.3	
10	21	0	0	3	2	0	0	0	5	33.3	
10, 2	25	0	0	1	0	1	0	0	2	13.3	4
10, 2	20	0	0	0	0	0	0	0	0	0.0	
10, 2	27	0	0	1	0	0	0	0	1	6.7	
10, 2	18	0	0	1	0	0	0	0	1	6.7	

positive effects of higher dietary fat levels obtained in previous studies (Lochmann and Phillips 2001). However, fish responses to bacterial challenge were not evaluated in those studies. Health effects of dietary fats depend on both the amount and type of fats used (Blazer 1992; Balfry and Higgs 2001) and vary according to species and the performance criteria evaluated.

The beneficial effects of the dairy-yeast prebiotic in the golden shiner are in agreement with those described by Li and Gatlin (2004, 2005) for hybrid striped bass, suggesting that prebiotics have the potential to replace antibiotics as prophylactic treatments to improve the resistance of fish to certain pathogens. As an added benefit, feed producers can purchase prebiotics without a veterinarian's prescription. The dairy-yeast prebiotic has the potential to increase the profitability of golden shiner production while limiting disease-related mortalities. The added cost of diets containing prebiotics will have to be weighed against the potential losses (mortalities) that producers experience when they use unsupplemented diets.

In addition, while disease challenges provide a model for determining fish health characteristics, they may not accurately reflect the conditions of disease outbreaks at production facilities. Therefore, the feasibility of prebiotic use to moderate disease susceptibility and to reduce mortality under different production conditions requires further evaluation. Growth of the golden shiners during the 16-week feeding period was slow, which is typical of this species in indoor aquaria. The effects of dairy-yeast prebiotics on growth and disease resistance in golden shiners need to be evaluated in an outdoor system more suitable for optimal growth of the species.

The actual mechanisms of action of prebiotics in species where efficacy is demonstrated need to be determined. We presume that positive modifications of the intestinal bacteria, as suggested by Li and Gatlin (2004, 2005), enhanced the survival of golden shiners exposed to columnaris, but we did not characterize the intestinal microflora in this study. Additional studies on the effects of prebiotics introduced at different life-stages of the fish and under different production scenarios would also be beneficial.

References

Aylward, N., and N. Bofinger. 2006. A plausible prebiotic synthesis of pyridoxal phosphate: vitamin B_6—a computational study. Biophysical Chemistry 123:113–121.

Balfry, S., and D. Higgs. 2001. Influence of dietary lipid composition on the immune system and disease resistance of finfish. Pages 213–234 *in* C. Lim and C. Webster, editors. Nutrition and fish health. Food Products Press, Binghamton, New York.

Blazer, V. 1992. Nutrition and disease resistance in fish. Annual Review of Fish Diseases 2:309–323.

Fracalossi, D. M., and R. T. Lovell. 1994. Dietary lipid sources influence responses of channel catfish to challenge with the pathogen *Edwardsiella ictaluri*. Aquaculture 119:287–298.

IIC (International Ingredient Corporation). 2006. GroBiotic®-A. Available: www.iicag.com/Grobiotic%20archive.php. (June 2005).

Li, P., and D. M. Gatlin III. 2004. Dietary brewers yeast and the prebiotic GroBiotic registered AE influence growth performance, immune responses, and resistance of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) to *Streptococcus iniae* infection. Aquaculture 231:445–456.

Li, P., and D. M. Gatlin III. 2005. Evaluation of the prebiotic GroBiotic®-A and brewer's yeast as dietary supplements for subadult hybrid striped bass (*Morone chrysops* × *M. saxatilis*) challenged in situ with *Mycobacterium marinum*. Aquaculture 248:197–205.

Lochmann, R., and H. Phillips. 1996. Stable isotope evaluation of the relative assimilation of natural and artificial foods by golden shiners (*Notemigonus crysoleucas*) in ponds. Journal of the World Aquaculture Society 27(2):168–177.

Lochmann, R., and H. Phillips. 2001. Nutritional aspects of health and related components of baitfish performance. Pages 119–130 *in* C. Lim and C. Webster, editors. Nutrition and fish health. Food Products Press, Binghamton, New York.

Lochmann, R. T., H. Phillips, K. Dabrowski, and R. Moreau. 2001. Responses of juvenile golden shiner *Notemigonus crysoleucas* fed semipurified or practical diets with or without supplemental ascorbic acid. Journal of the World Aquaculture Society 32(2):202–209.

Lochmann, R., N. Stone, and H. Phillips. 2004. Evaluation of 36%-protein diets with or without animal protein for indoor tank-hatched golden shiner fry in ponds. North American Journal of Aquaculture 66:271–277.

Moon, H. Y., and D. M. Gatlin III. 1991. Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95:97–106.

NASS (National Agricultural Statistics Service). 2006. Value of aquaculture products sold by type, by state and United States: 2005 and 1998. Available: www.nass.usda.gov/census_of_agriculture/2002/aquaculture/index.asp. (July 2007).

SPSS. 2000. SPSS 11.0 base system user's guide. SPSS, Inc., Chicago.

Thomas-Jinu, S., and A. E. Goodwin. 2004. Morphological and genetic characteristics of *Flavobacterium columnare* isolates: correlations with virulence in fish. Journal of Fish Diseases 27:29–35.

Vazquez, M. J., J. L. Alonso, H. Dominguez, and J. C. Parajo. 2006. Enhancing the potential of oligosaccharides from corncob autohydrolysis as prebiotic food ingredients. Industrial Crops and Products 24:152–159.