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[Technical Note]

Preliminary Observations of Mortality Reduction in Stressed, *Flavobacterium columnare*–Challenged Golden Shiners after Treatment with a Dairy-Yeast Prebiotic

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Abstract.—We conducted preliminary studies to evaluate the effects of including dairy-yeast prebiotics in the diets of golden shiners *Notemigonus crysoleucas* with access to natural foods on their resistance to challenge with *Flavobacterium columnare*. In trial 1, fish were fed either a control diet or a 2% dairy-yeast prebiotic diet for 10 weeks in outdoor pools before challenge. In trial 2, fish fed the experimental diets were either subjected to confinement stress or left unmolested before challenge. Mortality (mean ± SE) was not significantly different in the control diet (23.4 ± 3.4%), the prebiotic diet (10.0 ± 3.3%), and the prebiotic diet with stress (16.7 ± 3.4%) treatments. However, mortality was significantly greater in the control diet with stress treatment (50.0 ± 3.3%) than in the other treatments. This preliminary investigation suggests that prebiotic supplementation in golden shiner feeds before a stressful event would significantly reduce the mortality from *F. columnare*.

Prebiotic feed additives could benefit the immune systems of golden shiners *Notemigonus crysoleucas* and reduce the mortality due to *Flavobacterium columnare*. Dairy-yeast prebiotics effectively increased the survival of hybrid striped bass (striped bass *Morone saxatilis* × white bass *M. chrysops*; Li and Gatlin 2004, 2005) and golden shiners (Sink et al. 2007c) exposed to pathogens. Data from Sink et al. (2007c) indicate that dairy-yeast prebiotic feed additives decrease the susceptibility of golden shiners raised in indoor aquaria to *F. columnare* infections.

The growth of golden shiners in indoor systems is slow (Lochmann et al. 2001), and prepared diets are the only nutrient source (Lochmann and Phillips 1996). Growth is more rapid in production ponds (Lochmann et al. 2004) owing to the presence of natural foods, which golden shiners utilize for more than 40% of their nutritional requirements even when prepared diets are used (Lochmann and Phillips 1996). Natural food intake during pond production may dilute or counteract the growth of health-promoting bacteria in the intestinal tract that is mediated by prebiotics (Li and Gatlin 2004).

We conducted preliminary experiments on the effects of a dairy-yeast prebiotic on golden shiners raised in outdoor pools before challenge with *F. columnare*. The pools contained the same natural foods that are available during commercial pond production. In trial 1, golden shiners with access to natural foods were fed a prebiotic before exposure to *F. columnare*. As there is currently no literature on the interaction between stress and prebiotics in baitfish, a second trial was conducted in which golden shiners with access to natural foods were fed dairy-yeast prebiotics and either subjected to stress or left unmolested before exposure to *F. columnare*. The results of this study will provide an indication of the effectiveness of the dairy-yeast prebiotics used in commercial golden shiner production and could lead to further research on prebiotic use with baitfish.

Methods

Diets.—The composition of the two isonitrogenous diets (30.3 ± 0.3% crude protein [mean ± SE]) used in these studies is shown in Table 1. GroBiotic-A is a dairy-yeast prebiotic that contains yeast and dairy products as well as dried fermentation products that are high in oligosaccharides (IIC 2006). The diets were prepared as in Sink et al. (2007b) and kept frozen (–4°C) until use.

Fish.—Golden shiners ($N = 4,000$) from production ponds underwent a health inspection at the disease diagnostic laboratory at the University of Arkansas at Pine Bluff and were found to be free of clinical signs of disease before the trials. Four hundred fish (weight, 0.46 ± 0.02 g [mean ± SE]) were stocked into each of four replicate outdoor pools (2.7 m in diameter, 0.8 m deep) per diet. Pools were fertilized (10-30-0 [N–P–K] liquid fertilizer at 9.5 L/ha and cottonseed meal at 113.5 kg/ha) 2 weeks before stocking and then held static. The fish in each pool were fed one experimental diet to apparent satiation twice daily for 10 weeks. Twenty-five fish from each pool were then restocked into 110-L indoor tanks for the disease challenge (the individual weights were 1.6 ± 0.1 g for control fish

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TABLE 1.—Composition (%) of control and prebiotic diets fed to golden shiners in pools for 10 weeks before challenge with *Flavobacterium columnare*.

Ingredient (% as fed)	Control diet	Prebiotic diet
Menhaden Select fish meal	5.0	5.0
Poultry by-product meal	10.0	10.0
Soybean meal	43.0	43.0
Cottonseed meal	10.0	10.0
Corn	13.0	11.0
Wheat bran	13.0	13.0
Vitamin mix[a]	1.0	1.0
Mineral mix[a]	1.0	1.0
Poultry fat	4.0	4.0
Grobiotic-A[b]	0.0	2.0

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

and 1.8 ± 0.1 g for fish fed the prebiotic diet), where they continued to receive their respective diets. The fish were allowed to acclimate for 5 d before the challenge. The restocking process was repeated in the second trial.

Experimental system.—The experimental system consisted of eight 110-L glass flow-through (1.1-L/min; alkalinity, 19.2 mg/L; hardness, 25.9 mg/L) aquaria with external standpipes maintaining 85 L of water in each tank. An air stone was supplied to each tank to provide supplemental aeration.

Flavobacterium columnare.—A virulent strain of *F. columnare*, PB02–41 (Thomas-Jinu and Goodwin 2004), was prepared as in Sink et al. (2007c). The isolate reached an optical density of 0.410 absorbance (0.429 absorbance for trial 2) at 560 nm before use in the challenges. Bacterial concentration exposures were previously determined from trials with golden shiners to determine the dosages lethal to 30% of the fish (LD30 trials).

Disease challenge.—In trial 1, water flow to the tanks was shut off and aeration was maintained. Twenty milliliters of *F. columnare* in Sheih broth was added to each aquarium for an 18-h exposure. Water flow was then restored, and the *F. columnare* were flushed from the system. Mortality was then monitored and recorded for 10 d (Sink et al. 2007c).

This procedure was repeated in trial 2, except that the golden shiners from two control and two prebiotic tanks were captured and held in nets within the tanks for 30 min (i.e., subjected to confinement stress) before exposure to *F. columnare*. The fish in the remaining two tanks for each diet were left undisturbed.

Stress and cortisol analysis.—To demonstrate that the fish in trial 2 were sufficiently stressed by the confinement treatment, four 110-L glass aquaria were later stocked with 30 golden shiners (weight, 1.0 ± 0.03 g). The fish were allowed to acclimate for 3 d while receiving the control diet. Five fish from each tank were euthanized with 200 mg tricaine methanesulfonate/L and frozen at −70°C for cortisol analysis. The remaining fish in each tank were subjected to stress as in trial 2. At the conclusion of the stress, five fish from each tank were euthanized with 200 mg tricaine methanesulfonate/L and frozen at −70°C until cortisol analysis was conducted.

Cortisol assays were conducted with whole-body extract as in Sink et al. (2007a), with the following modifications: After each golden shiner was weighed, it was sliced into small sections and placed directly into a disposable 10-mL screw-top test tube with 1 mL of phosphate-buffered saline. The fish was then homogenized by maceration with a clean 8-mm-diameter glass rod. One hundred microliters of previously assayed food-grade vegetable oil was added per gram of body weight. Four milliliters of ethyl ether was then added to the tube, and the tube was capped. The remaining procedure was the same as in Sink et al. (2007a). An enzyme-linked immunosorbent cortisol assay (Assay Designs, Ann Arbor, Michigan; kit 900–071) was conducted as validated for use in golden shiners by Sink et al. (2007b).

Statistical analysis.—The percent mortality and cortisol concentrations of the golden shiners in each treatment were analyzed with a one-way analysis of variance (ANOVA) and Tukey's post hoc test in SPSS 11.0 to determine differences between treatments. Because the data had a range of more than 40%, they were arcsine-transformed to meet the normality assumptions of the ANOVA. Differences were considered significant at $P < 0.05$.

Results and Discussion

In the LD30 trials, the first mortalities occurred within 2–3 d, the majority occurred within 5–7 d, and they tapered off within 8–9 d. For this reason, mortality was monitored for only 10 d in the present study. Mortality ranged from 8% to 24% and was not significantly different ($P < 0.05$) for fish fed the control (18.0 ± 1.2%) and prebiotic (15.0 ± 3.4%) diets in trial 1 (diet only). In trial 2 (diet with or without confinement stress), mortality was not significantly different in the control diet (23.4 ± 3.4%), prebiotic diet (10.0 ± 3.3%), or prebiotic diet with stress (16.7 ± 3.4%) treatments. Mortality in the control diet with stress treatment (50.0 ± 3.3%) was significantly ($P = 0.004$) greater than that in the other treatments.

Net confinement induced a significant stress response. Golden shiners subjected to 30 min of confinement exhibited significantly ($P = 0.001$) higher cortisol concentrations than control fish (51.2 ± 5.6 versus 23.7 ± 2.3 ng cortisol/g).

The results from trial 1 conflict with those of Sink et al. (2007c) in indicating that diets containing dairy-yeast prebiotics do not reduce the susceptibility of golden shiners to *F. columnare*. However, the latter study was conducted in indoor tanks in which the experimental diets were the sole source of nutrients for the fish (Lochmann and Phillips 1996). It is possible, therefore, that the effects of dietary supplements were diluted in golden shiners that had access to natural foods. This does not appear to be the case, however; if it were, we would expect to see mortality rates similar to those of the control treatment (41.7 ± 12.9%) in Sink et al. (2007c) in both of the treatments in the present study. Instead, the golden shiners appeared to be healthier when raised in an outdoor system, as indicated by the low mortality rates for both treatments in the present study (15% and 18%).

Based on the results of trial 1, one must reject the hypothesis that the use of prebiotics in the diets of golden shiners raised outdoors improves the survival of fish exposed to *F. columnare*. However, the results of trial 2 justify the use of prebiotics in golden shiner diets under some circumstances; even when natural foods were available, golden shiners that were fed diets supplemented with prebiotics before crowding stress experienced significantly lower mortality when exposed to *F. columnare* than those fed control diets.

The use of dairy-yeast prebiotics before stressful events such as harvest, grading, and shipping could decrease mortality rates in golden shiners exposed to pathogens and increase the profitability of golden shiner production. In this study, the diet containing prebiotics was fed for weeks before a known stressful event. In production, the added cost of supplemented diets will have to be weighed against the losses that producers might experience.

This pilot study did not show increased survival among golden shiners exposed to a particular pathogen, but it offers a useful insight into the interaction between prebiotics and stress that has not been reported in previous research. The results of these trials both provide a basis for the selective use of prebiotics in baitfish culture and demonstrate the need for further research on their physiological effects and production potential.

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