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Immunization of Channel Catfish, *Ictalurus punctatus*, Against Two Bacterial Diseases

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ABSTRACT—A field study was begun to compare the efficacy of three different routes of immunization of channel catfish, Ictalurus punctatus, *intensively cultured in cages. A polyvalent bacterin against* Aeromonas hydrophila *and* Flexibacter columnaris *was administered by oral, injection, and immersion routes. Initial data indicated a humoral antibody response to all three routes. Thirty-day post-immunization agglutinin titers were as high as 1:1,280 in fish injected with bacterin, 1:160 in immersion-treated fish, and 1:20 in orally immunized fish. Gut mucosal extracts of identical fish had precipitin titers as high as 1:5,120 in immersed fish, 1:320 in orally immunized fish, and 1:160 in injected fish. Control titers were 1:80. No significant difference in protective immunity was detected. These data reflect initial results of a 2½-year study which will be reported in full at a later date.*

During recent years, cultural and nutritional studies with channel catfish, *Ictalurus punctatus*, at the Fisheries Research Unit at Auburn University have been plagued with bacterial disease problems. Epizootics of *Aeromonas hydrophila* and *Flexibacter columnaris* have been particularly troublesome in intensive culture techniques such as in pens and cages. These bacteria are also of importance in open pond culture, particularly under poor environmental conditions.

Experiments conducted at Auburn in 1972 indicated that the channel catfish would produce high circulating antibody titers when injected with a heat-killed bacterin. Additional studies also revealed that agglutinin titers as high as 1:160 could be induced following a single immersion treatment of bacterin.

METHODS OF TEST IMMUNIZATIONS

During the spring of 1974 a study was begun to test the possibility of immunizing channel catfish in cages against *A. hydrophila* and *F. columnaris*. The experiment was designed to test the level of protective immunity by observations of survival following natural infection with these bacteria in a pond. The experimental pond had a 5-year history of epizootics of these organisms in the cage culture of catfish. Differences in response of fish to three routes of administration of the bacterin were also measured by survival data, titering fish sera, and mucosal samples for circulating and secretory antibody. Twenty-four hundred channel catfish fingerlings were selected and divided into four groups of 600 fish, each group in a 730-liter tank. Three 600-fish groups were designated for bacterin administration by either injection, immersion, or oral routes, and the remaining group served as a control. A heat-inactivated polyvalent bacterin with adjuvant was prepared with *A. hydrophila* and *F. columnaris*. Injected fish received a single 0.2 ml intramuscular dose of bacterin with adjuvant while the immersion treatment consisted of the addition of bulk vaccine directly to the tank such that the final dilution was 1/126. Fish fed vaccine were administered treated feed every other day for a total of six treated rations; controls were held under identical conditions without bacterin administration. Following a suitable period for response to the bacterin, all fish were stocked randomly by treatment into 12 respective 0.75-m^3 cages anchored on a line in a 4.8-acre pond.

At 30 days postimmunization, a subsample was removed from each treatment group and the control group. Serum, surface mucus, and gut mucosal samples were collected and titered using microtiter techniques. Serum was titered against *A. hydrophila* and *F. columnaris* whole cell antigens, while mucosal extracts were tested against sonicates of the same organisms using a capillary tube precipitin technique.

RESULTS OF IMMUNIZATION

Following the 5-month experimental period, during which infections of both bacteria were diagnosed, the fish were counted for survival data. Sixty-eight percent of the controls survived, while 66 percent of the orally treated, 78 percent of the immersion treated, and 80 percent of the injected fish survived. Thirty day postimmunization agglutinin titers of injected fish were as high as 1:1,280, 1:160 in immersion-treated fish, and only 1:20 in feed-treated fish. Precipitin titers in gut mucosal extracts from the same fish were as high as 1:5,120 in immersed fish, 1:320 in feed-treated fish, and 1:160 in injected fish. Controls had a titer of 1:80 which was considered to be a result of exposure prior to the experiment. Statistical

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analysis of survival data revealed no significant differences ($P \geq 0.05$) between treatment groups and the control group or among treatment groups. This was thought to be a result of a high degree of variation encountered in the cages. However, percentage values seem to indicate that the immersion and injection routes might give significant results with further studies. The fact that there is a circulating and secretory antibody response to the immersion route seems to indicate that further refinement of this technique may result in an effective immunization method which will eliminate individual handling of each fish.

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