organisms. The production of intracellular superoxide anion as well as the extracellular secretion of superoxide anion and hydrogen peroxide can be measured.

It was found that vaccination enhanced the ability of macrophages to produce intracellular superoxide anion after phagocytosis of live E. ictaluri. The production was increased 8.5x in the fish fed combo feed, 6.5x in fish fed the commercial feed and only 2.3x in fish fed the beef tallow feed. The extracellular secretion of superoxide anion and hydrogen peroxide was increased in vaccinated fish from the combo and commercial groups but not in the fish fed beef tallow.

The conclusion is that nutritional manipulation can be used to potentiate the immune response. Certain lipids appear to be very useful in the immune response and macrophage function. A combination of menhaden oil, soybean oil and beef tallow is probably the best regarding both disease resistance and growth. If lipid is used as a dietary enhancement prior to vaccination, these feeds would have to be fed for 3-4 weeks (at 24°C or above) prior to vaccination.

USEFULNESS OF FINDINGS:

This work indicates that a combination of dietary immunopotentiators and vaccination programs could significantly reduce losses due to E. ictaluri.

PUBLICATIONS:

Vinitnantharat, S. 1991. Humoral and cell-mediated immune response of channel catfish, Ictalurus punctatus, to Edwardsiella ictaluri. Ph.D. Dissertation, Auburn University, AL. 155 pp.

Plumb, J. A. and S. Vinitnantharat. 1991. Kinetics of the immune response in channel catfish to Edwardsiella ictaluri. 16th Annual Eastern Fish Health Workshop. Martinsburg, WV. June, 1991.

Hanson, L. A. 1990. Biochemical characterization and gene mapping of the channel catfish herpesvirus (CCV) encoded thymidine kinase, a selectable site for homologous recombination. Ph.D. dissertation. Louisiana State University, Baton Rouge, LA. 70803.

Awad, M. and R. L. Thune. 1991. Cloning and expression of the S-layer protein gene of Aeromonas hydrophila. Proceedings of Annual Meeting of Fish Health Section of American Fisheries Society, Portland, OR. p. 29.

Lingenfelser, J. T., V. S. Blazer and R. E. Klinger. 1991. Metabolic activation of channel catfish macrophages. 16th Annual Eastern Fish Health Workshop. Martinsburg, WV. June, 1991.

B. Enhancement of the Immune Response to Edwardsiella ictaluri in Channel Catfish

Termination Report
For the Period
May 2, 1989 - September 30, 1991

COOPERATING INSTITUTIONS:

Clemson University (Lead Institution) - J. R. Tomasso and T. E. Schwedler, Department of Aquaculture, Fisheries and Wildlife

Texas A&M University - D. M. Gatlin and W. H. Neill, Department of Wildlife & Fisheries Sciences; D. H. Lewis, School of Veterinary Medicine

University of Georgia - Vicki S. Blazer, Georgia Cooperative Fish & Wildlife Research Unit

ADMINISTRATIVE ADVISOR:

J. R. Fischer, Director
South Carolina Agric. Experiment Station
Clemson University
Clemson, South Carolina

REASON FOR TERMINATION:

Project completed.

PRINCIPAL ACCOMPLISHMENTS:

Effect of Selenium on the Immune Response

Purified diets containing adequate vitamin E (60 IU/kg) were supplemented with 0, 0.25 and 10 mg/kg Se and fed to immunized and non-immunized fingerling catfish in aquaria to evaluate the effects of dietary selenium on immunocompetence and disease resistance to E. ictaluri. At the end of the 15-week feeding trial, selenium status of fish fed the various diets was confirmed by analysis of selenium-dependent glutathione peroxidase (SeGSH-Px) activity in liver. Fish fed the basal diet were selenium deficient as evidenced by significantly ($P < 0.05$) reduced SeGSH-Px activity as compared to fish fed diets supplemented with 0.25 and 10 mg/kg Se. However, supplemental selenium in the diet did not improve immunocompetence of catfish based on assessment of antibody titers, phage neutralization, peritoneal macrophage activity and resistance to a challenge by live E. ictaluri. In fact, selenium deficiency actually improved the resistance of catfish to bacterial challenge. Similar responses have been observed in some selenium-deficient mammalian species.

Subsequent experiments to evaluate the combined effects of dietary selenium and vitamin E on immunocompetence of channel catfish are proposed since these nutrients have complementary biochemical functions which may interact synergistically.

Effect of Vitamin E on the Immune Response

Channel catfish fingerlings were acclimated to laboratory conditions and fed diets containing 0, 60 or 2,500 IU/kg vitamin E for 3.5 months. Half of the fish in each treatment were immersion vaccinated after 0.5 months using formalin-killed E. ictaluri. These fish also received an oral booster two months later.

After 3.5 months, the vaccinated fish had a significantly higher phagocytic index than the non-vaccinated fish (2-way ANOVA). Phagocytic index in the immunized fish also increased significantly in a diet-dependent manner in the vaccinated groups (1-way ANOVA). Bactericidal activity was significantly affected by diet in both vaccinated and non-vaccinated groups (1-way ANOVA); however, no pattern was evident. In general, bactericidal activity was higher in the non-vaccinated groups (2-way ANOVA). The groups (both vaccinated and non-vaccinated) fed the high vitamin E diet were significantly more resistant to red blood cell peroxidation than the groups fed the intermediate and low vitamin E diets. All groups responded similarly to challenge by injection of live bacteria.

Effect of Levamisole on the Immune Response

Initial studies revealed that bath immunization of catfish fingerlings and adults with formalin-killed-bacterins of E. ictaluri did not yield consistent immune responses with respect to protection and various serologic parameters. Hence, a pilot study was conducted comparing various formalin- and heat-killed preparations for efficacy in bath immunization of catfish. The bacterin which proved superior was prepared from a two broth culture, washed 2x in saline, autoclaved and the turbidity adjusted to Macfarland standard 4, and diluted 10x in a bath wherein fish were held for 20 minutes. Four groups of fish were studied, i.e., a control group maintained on

a conventional diet containing no Se, a group maintained on 1% Carrisyn (an immunopotentiator), and a group which received levamisole after having been immunized. Each of the four groups were subdivided into subgroups, one of which had been immunized and the other which had not been immunized. All the fish which were immunized had significant agglutinin titers two weeks after immunization; the agglutinin titers of those fish which had received Carrisyn were 2- to 8-fold higher than control fish; levamisole treatment and selenium deprivation enhanced serologic response approximately 2-fold. Challenge studies using 2XLD50 live organisms revealed that selenium deprivation and the incorporation of Carrisyn enhanced protectiveness of the immunization protocol.

Effect of Combinations of Vitamin E and Selenium on the Immune Response

Channel catfish were fed five diets containing combinations of selenium and vitamin E (0 IU/kg E and 0 mg/kg selenium; 60 IU/kg E and 0 mg/kg selenium; 0 IU/kg E and 0.25 mg/kg selenium; 240 IU/kg E and 1.0 mg/kg selenium). Fish were fed the experimental diets for at least 120 days. Half of the fish receiving each diet were vaccinated by immersion in 5×10^9 formalin-killed cells per ml on day 90, given oral boosters (15.7×10^{10} formalin-killed cells/kg) on days 104-106, and sampled beginning on day 120. Production of intracellular and extracellular superoxide anion by macrophages, glutathione peroxidase activity of liver, red blood cell resistance to peroxidation, and resistance to challenge by live bacteria were determined. In all treatments except the double deficient group, immunization significantly enhanced the intracellular production of superoxide anion subsequent to phagocytosis of E. ictaluri. The two selenium-deficient groups produced the lowest amounts of superoxide anion in both vaccinated and non-vaccinated groups. Extracellular superoxide anion in both vaccinated and non-vaccinated groups was lowest in the double deficient group. Glutathione peroxidase activity was lowest in the two selenium deficient treatments, highest in the high selenium treatment, and unaffected by vaccination status. Fish fed the two vitamin E-deficient diets were more susceptible to red blood cell peroxidation than fish fed the remaining three diets. No significant differences were observed in the challenge studies.

Effect of Levamisole, Cortisol and Stress on Immune Response

Experimental design of this study involved five vaccinated treatments in duplicate (total of 10 groups, 20 fish/group): (1) control; (2) levamisole for four days prior to initial sampling; (3) cortisol (1700 mg/kg) two weeks prior to initial sampling; (4) cortisol + levamisole and (5) Carrisyn. Immunologic assays were conducted five weeks after initiating the study. Mean plasma cortisol concentration of fish not receiving cortisol was 8.2 ng/ml, while that of fish receiving cortisol was 163.0 ng/m. Pronounced immunosuppression was observed in fish receiving cortisol in that none of these vaccinated fish developed agglutinins during the study. In fact, of the cortisol treated fish, 4/10 fish in one replicate group and 7/10 in the other succumbed before the study was terminated. The ameliorating effect of levamisole in counteracting the immunosuppressive effect of cortisol was not statistically significant ($p < 0.05$). The resistance enhancing effects of Carrisyn were further verified in that bactericidal and phagocytic assays were enhanced by a factor of 2-3 fold over control fish which had agglutination titers of 1:64-1:256. Eighty-two percent of the fish survived bacterial challenge. None of the fish which had received Carrisyn succumbed to challenge.

In an effort to ascertain the potential ameliorating effects of levamisole and Carrisyn upon stress-induced immunosuppression, the fish from one replicate of each treatment were stressed

by placing in nets just below the surface of the water for 24 hours. Three fish in each tank were processed for: a) phagocytic assays, b) bacterial challenge, c) phage neutralization and d) plasma cortisol assay prior to and immediately after imposing the 24-hour stress test. Across all treatments, stressed fish possessed a mean cortisol level of 93 ng/ml while unstressed fish possessed a mean cortisol level of 11 ng/ml. Phagocytic assays and other indicators of immune responsiveness were higher in levamisole treated, vaccinated stressed fish, than in those vaccinated stressed fish which had not received levamisole. However, there was no difference in resistance to challenge between stressed and non-stressed levamisole-treated fish. Approximately 40% of the stressed fish which had been immunized ultimately succumbed to bacterial challenge whether or not they had received levamisole. The immunopotentiating effects of Carrisyn were not observed in stressed fish in that there appeared to be no difference in survival to challenge or other immunoassays between fish receiving Carrisyn and those not receiving Carrisyn.

USEFULNESS OF FINDINGS:

The findings of this research project will be useful in:

(1) designing diets to help promote immunity to E. ictaluri;

(2) designing diet/vaccination regimes for promoting immunity to E. ictaluri; and

(3) designing further studies to develop techniques to more efficiently immunize channel catfish against E. ictaluri.

PUBLICATIONS:

Blazer, Vicki S. 1991. Piscine macrophage function and nutritional influences: A review. Journal of Aquatic Animal Health 3:77-86.

C. Effect of Nutrition on Body Composition and Subsequent Storage Quality of Farm-Raised Channel Catfish

Annual Progress Report
For The Period
October 1, 1990 to September 30, 1991

COOPERATING INSTITUTIONS:

Auburn University (Lead Institution)	
Fisheries	R.T. Lovell
Ag. Economics	Upton Hatch
Kentucky State University	
Aqua. Res. Center	J.H. Tidwell
	C. Webster
Louisiana State University	
Forestry, Wildlife & Fisheries	R.C. Reigh
Food Science	J. S. Godber
Mississippi State University	
Delta Research and Extension Center	E.H. Robinson
Biochemistry	R.P. Wilson
Wildlife & Fisheries	H.R. Robinette
Agric. Economics	J.E. Waldrop
Food Science & Human Nutrition	J. Hearnsberger
Texas A & M University	
Wildlife & Fish. Science	D.M. Gatlin
University of Georgia	
Food Science & Tech.	J.J. Jen
	Y.W. Huang
	D.A. Lillard
	P.E. Koehler
	R. EitenMiller
Georgia Exp. Station, Griffin, Ga.	M. Erickson
Coastal Plains Exp. Station, Tifton, Ga.	G. Burtle