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Vaccination of Nile Tilapia (*Oreochromis niloticus*) Using Lipopolysaccharide (LPS) Prepared from *Aeromonas hydrophila*

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Abstract
The efficacy of *Aeromonas hydrophila* lipopolysaccharide (LPS) as a vaccine was studied in Nile tilapia (*Oreochromis niloticus*). Vaccine efficacy was measured in terms of the Relative Percent Survival (RPS). The fish was immersed in the vaccine at different duration of exposure. To compare the protective effect of the vaccine, *O. niloticus* was challenged intraperitoneally with 0.1mL of virulent *A. hydrophila* suspension at 10^6 cells/mL.
The RPS value of the vaccinated group at 120 sec. immersion was the highest. The results confirm the efficacy of the LPS vaccine prepared from *A. hydrophila* and showed that the higher the immersion time, the greater was the efficacy of the vaccine.

Keywords: Tilapia, *Aeromonas hydrophila*, Vaccine

1. Introduction
Aquaculture is the farming of desirable aquatic organisms in ponds, recirculating tanks and cages. Fish production through aquaculture contributes to improve the condition of poverty and malnutrition in Asia. However, culture operation has led to major ecological strain on the aquatic environment and also cause stress to fish. Both eventually lead to lower productivity and outbreak of diseases.
Bacterial diseases are among the most serious infectious problems of cultured tilapia. One of the most common bacterial disease of tilapia is Motile Aeromonas, Septicemia (MAS) caused by *Aeromonas hydrophila* and related species. Tilapia with MAS loses their equilibrium, swim lethargically and gasp at the surface. Clinical signs include fray and haemorrhages. *A. hydrophila* also caused septicemic disease on cultured Nile tilapia (Yambot and Inglis, 1994 [1]; Plumb, 1997 [2]; Yambot, 1998 [3]).
The best prevention and treatment for MAS is good husbandry. However, it is not possible to improve conditions when resources are being used near their limit. In these situations, antibiotics, particularly oxytetracycline or the nitrofurans, are used for both treatment and prophylaxis. But the extensive use of antibiotics has the serious drawback of increasing plasmid-encoded antibiotics resistance in *A. hydrophila*. Concerns about extensive use of antibiotics in aquaculture make vaccine against *A. hydrophila* an attractive option when there is limited flexibility to improve environmental and management conditions (Stevenson, 1988) [4]. The vaccine is a preparation of killed micro-organisms which are attenuated, fully virulent or nonvirulent. It is capable of causing the antibody production against an infectious micro-organism when artificially introduced into the body, thereby, conferring immunity from a subsequent infection of that micro-organism. Once stimulated by a vaccine the antibody-producing B-cells of the body remain sensitized to the infectious agent and respond to reinfection by producing more antibodies, thus reinstituting the immune response (Gwinn, 1991) [5].
Crude lipopolysaccharide (LPS) with its characteristic of heterogeneity, increases antibody titers and enhances total blood phagocytosis activity. It is a good immunogen, easier to prepare and suitable for the preparation of vaccines (Salati, 1988) [6].

2. Materials and Method
2.1 Source and Acclimation of Nile Tilapia
Oreochromis niloticus with size ranging from 40-60g were obtained from the Freshwater

Aquaculture Center-College of Fisheries, Central Luzon State University. Ten fish were stocked in each aquarium with a dimension of 12 x 24 x 12 inches and acclimatized for one day. The volume of the water was 30 L in each aquarium. The fish were fed with prepared diet, which composed of rice bran (D1) (67%) and fish meal (33%). Feeding rate was 7% of the body weight of the fish per day. Feeding amount per day computed from the feeding rate was divided into three and given at 8 am, 12 noon and 4 pm. Aerators were provided and a daily change was done.

2.2 Experimental design

A completely randomized design (CRD) was used in setting-up the experiment. The five treatments were replicated three times described as follows:

T1 - fish without the application of vaccine
T2 - fish immersed in crude LPS vaccine for 30 seconds
T3 - fish immersed in crude LPS vaccine for 60 seconds
T4 - fish immersed in crude LPS vaccine for 90 seconds
T5 - fish immersed in crude LPS vaccine for 120 seconds

2.3 Preparation of Crude Lipopolysaccharide (LPS) Vaccine

Five 500 mL (in 500 mL flasks) of tryptic soy broth inoculated with *Aeromonas hydrophila* were used as culture media to grow the cells for 24 hours. The bacterial cells were collected from the broth and the crude LPS was extracted according to the procedure described by Anderson (1992) [7].

Briefly, the culture *A. hydrophila* was centrifuged at 1600 rpm for twenty minutes and was suspended in saline, ten times the volume of the resulting product. The bacterial cells were suspended for two hours at 100 ^{o}C. The cells were then centrifuged and the supernatant was discarded. The cells were then resuspended in 95% ethyl alcohol, ten times more fluid than the cell packed and were incubated at 37 ^{o}C for four hours. The cells were then washed with acetone and were ground to produce a fine powder.

The vaccine solution was prepared by dissolving the extract in 30,000 mL of water.

2.4 Administration of Vaccine

The *O. niloticus* test species were immersed in the vaccine at different duration of immersion following the experimental design after the seven days of feeding. Administration of vaccine by immersion of fish was done.

2.5 Challenge test

Two weeks after vaccination, each fish was injected intraperitoneally (IP) with 0.1 mL of *A. hydrophila* suspension at 10^6 cells/mL. During the four days challenge test, dissolved oxygen and temperature were monitored at 24th, 48th, 72nd and 96th hour.

2.6 Data Gathering

After challenging the Nile tilapia with the virulent *A. hydrophila* through IP injection, the mortality of the fish was monitored daily.

2.7 Data Analysis

Immunity against *A. hydrophila* was expressed in terms of Relative Percent Survival (RPS) as described by Ellis (1988).

$$\textbf{RPS} = 1 - \frac{\text{\% mortality vaccinated fish}}{\text{\% mortality of control}} \times 100$$

3. Results and Discussion

3.1. Relative Percent Survival

The results revealed that Treatment 5 had the highest RPS value of 58% followed by Treatment 4, Treatment 3, and Treatment 2 with RPS values of 45%, 36%, and 27%, respectively. Treatment 5 using fish immersed in the vaccine for 120 sec attained the highest level of protection against virulent bacterial pathogen. As stated by Morales (1995) [8] the higher the RPS value, the higher the level of protection.

Immersion of the fish in the vaccine gives protection against bacterial infection. Immersion vaccination could elicit a good immune response in fish as demonstrated by its increasing agglutinin titer and prolonged stimulation of the immune system. More fish can be likewise immunized by a given amount of vaccine. Furthermore, the time of immersion is also considered in the application of vaccine. Based on the result of the study, the longer the time of exposure to the vaccine the higher was the survival of fish. Thus, the vaccination through immersion had increased efficiency at the time was prolonged. As stated by Baba *et al*. (1988) [2] two hours immersed in the vaccine is more effective.

Immersion in the crude lipopolysaccharide vaccine has proved very successful in the vaccination trials as evidenced by the RPS value. Though little is known about the mechanism of vaccine uptake, the presentation of the antigen through the vaccine elicited immune response (Ellis, 1985) [11]. Furthermore, the procedure using immersion route was simpler and less stressful resulting to a higher degree of protection acquired (Baba *et al*., 1988) [2]. Therefore, immersion route appears more suitable for application in aquaculture in large scale vaccination and simple dipping is adequate (Scott, 1993) [9].

As stated by Plumb (1992) [7], vaccination is a disease prevention measure which is becoming more prominent in aquaculture. This practice widely used in veterinary and human medicine and it is applicable to aquatic animals. Immersion and oral application of vaccines appeared to be the most popular and logistically feasible methods of vaccinating fish.

When a fish is immersed in a vaccine, the vaccine is picked up by macrophages in the gill and delivered via the blood system to melanomacrophage centers in the spleen, kidney, and epicedium. Lymphocytes are then exposed to the antigen to begin production of a cell mediated (T cell) or antibody mediated (B cell) response. The antibodies specific for that antigen are produced as a primary response. Hence, during the actual attack of the living versions of the pathogen, secondary response of the immune system is quicker because the antigen has already been identified through the attenuated or killed antigen used as a vaccine.

In the experiment of Tatner and Horne (1983) [12], protective immunity of rainbow trout against *Vibrio anguillarum* was acquired after bath vaccination with a suspension of weakened bacteria. Furthermore, their study revealed that strains of *V. anguillarum* have antigenic *Vibrio* determinants which are common with *Aeromonas* strain. Therefore *Aeromonas* bacterin is bacteria are possible.

Fish as a biological organism possesses an innate mechanism for developing resistance to microbial organism. As such the study of immune response of fish has been continually gaining interest with

the end goal of vaccine development. Thus, vaccines are tools that exploit the ability of the fish to prevent foreign organisms from invading the host and disrupting health.

3.2 Water Quality Parameters

3.2.1 Dissolved Oxygen

The recorded DO readings in all treatments during the post-bacterial challenge ranged from 5.90 to 7.04 mg/L. Statistical analysis on the average DO showed no significant difference among the treatments.

3.2.2 Water Temperature

The recorded water temperature in all treatments ranged from 25.20 to 27.2 ^{0}C. Statistical analysis showed no significant difference among the treatments.

Sudden rise or fall on water temperature is a direct stress. Thus, the survival rate and the ability to combat disease are much lower outside the optimum range of the fish. High temperature also results in a fall in dissolved oxygen in the water, which may cause respiratory distress. This situation can lead to acute mortalities and highlights the complex relationship between the environment and the manifestation of disease. Thus, a sudden temperature change can precipitate outbreaks of infectious disease, perhaps because the pathogen adopts more rapidly than the immune system of the fish to the changes in temperature (Southgate, 1995).

4. Conclusion

Based on the results of the study, *O. niloticus* can acquire protection against the disease caused by *A. hydrophila* through vaccination using crude lipopolysaccharide prepared from *A. hydrophila*. The results also revealed that vaccination by immersion tend to protect the fish and the longer the time of exposure resulted in high relative percent survival. Therefore, the crude lipopolysaccharide vaccine can be used in the prevention of disease when adverse environmental condition is expected such as low temperature during cold months to prevent economic loss.

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