## IMMUNE RESPONSE OF NILE TILAPIA (Oreochromis niloticus) ON Aeromonas hydrophila ISOLATED FROM TAAL LAKE

### THESIS

**JENIELYN VELASQUEZ PONIENTE** 

COLLEGE OF ARTS AND SCIENCES
CAVITE STATE UNIVERSITY
Madenay, Cavita

April 2002

### IMMUNE RESPONSE OF NILE TILAPIA (Oreochromis niloticus) ON Aeromonas hydrophila ISOLATED FROM TAAL LAKE

Undergraduate Thesis
Submitted to the Faculty of the
Cavite State University
Indang, Cavite

In partial fulfillment of the requirement for the degree of Bachelor of Science in Biology (Major in Microbiology)



Immune response of Nile Tilapia (Oreochromis niloticus) on Aeromonas 639.8 P77 2002 T-2389

JENIELYN VELASQUEZ PONIENTE APRIL 2002

#### ABSTRACT

PONIENTE, JENIELYN VELASQUEZ, April, 2002, "Immune Response of tilapia (Oreochromis niloticus) on Aeromonas hydrophila Isolated from Taal Lake", An Undergraduate Thesis, Bachelor of Science in Biology, major in Microbiology, Cavite State University, Indang, Cavite. Advisers: Mr. Francisco M. Heralde III and Dr. Yolanda A. Ilagan

Immune response of Nile tilapia (*Oreochromis niloticus*) on *Aeromonas hydrohila* isolated from Taal Lake was conducted. This study aimed to: optimize the conditions for tilapia blood sample extraction and processing, antibody quantification and white and red blood cells detection and quantification and to determine the effect of *Aeromonas hydrophila* infection on the tilapia antibody titers and levels of white and red blood cells, physical appearance and mortality rate. Four treatments were employed: sterile distilled water as control, heat-killed bacteria, formalin- killed bacteria and bacteria suspended in saline solution at a rate of 2.15 x 10<sup>5</sup> cells per ml.

It was found out that 10*u*l of inoculum per gram of fish can be used as a standard concentration of antigen injected to fish considering their body weight.

Positive agglutination was observed in Nile tilapia after injection of *Aeromonas hydrophila*. Increase in antibody titer of fish at all treatments was detected on the third day of post- inoculation up to ten days. Rapid decline of antibody production was observed on the tenth day of post- inoculation and onwards.

Red blood cell (RBC) of Nile tilapia at different treatments except for the control declines after six days post- inoculation Decrease in leukocyte count of fish given heat-killed, formalin-killed and live bacteria suspended in 0.85% saline solution was observed

after stimulation of antigen.

During the conduct of the study, all fish samples exhibited symptoms of disease caused by *Aeromona hydrophila* such as exopthalmia, fin rot and lost of scales.

#### TABLE OF CONTENTS

	Page
BIOGRAPHICAL SKETCH	iii
ACKNOWLEDGMENT	iv
ABSTRACT	vi
TABLE OF CONTENTS	viii
LIST OF TABLES	X
LIST OF FIGURES	xi
LIST OF APPENDIX TABLES	xii
INTRODUCTION	1
Importance of the Study	2
Objectives of the Study	3
Time and Place of the Study	3
REVIEW OF RELATED LITERATURE	4
METHODOLOGY	16
Preparation of Materials and Media	16
Maintenance of Bacterial Strain.	16
Optimization of Bacterial Concentration	16
Experimental Set-up	17
Preparation of Bacterial Suspension.	17
Injection of Inoculum	18
Blood Extraction and Processing	18

Blood Monitoring	18
Bacterial Agglutination Assay	21
RESULTS AND DISCUSSION	25
SUMMARY, CONCLUSION and RECOMMENDATION	39
BIBLIOGRAPHY	41
APPENDICES	48

#### LIST OF TABLES

Table		Page
1	Antibody Titer of Nile tilpia (Oreochromis niloticus) before introduction of Aeromonas hydrophila	26
2	Antibody titer of Nile tilapia (Oreochromis niloticus) three days after introduction of antigen	27
3	Antibody titer of Nile tilapia (Oreochromis niloticus) six days after introduction of antigen	27
4	Antibody titer of Nile tilapia (Oreochromis niloticus) ten days after introduction of antigen	27
5	Antibody titer of Nile tilapia (Oreochromis niloticus) fifteen days after introduction of antigen	28
6	Average antibody titer of Nile tilapia (Oreochromis niloticus) at specific time interval	28
7	Mortality rate of Nile tilapia after fifteen days post- ntroduction of Aeromonas hydrophila	30
8	Mean red blood cell count of Nile tilapia from 0-15 days post- inoculation of <i>Aeromonas hydrophila</i>	32
9	Average number of white blood cell of Nile tilapia (Oreochromis niloticus)	37

#### LIST OF FIGURES

Figure		Page
1	Tilapia showing the site of inoculum injection	19
2	Fish showing site of blood extraction	20
3	Hemacytometer showing chambers where (a) red blood (b) white blood cell count were counted	22
4	Micro titer plate showing (a) positive agglutination and (b) negative agglutination	24
5	Mean antibody titer of Nile tilapia pre- after fifteen days post- inoculation of <i>Aeromonas hydrophila</i>	29
6	Red blood cells of tilapia (Oreochromis niloticus)	33
7	Mean red blood cell count of Nile tilapia from 0-15 days post- inoculation of <i>Aeromonas hydrophila</i>	35
8	White blood cells of tilapia (Oreochromis niloticus)	36
9	Mean white blood cell count of Nile tilapia from 0-15 days post-intoculation of Aeromonas hydrophila	38

#### **APPENDICES**

Appendix	: 1	Page
1	Composition and Preparation of Media and Reagents	49
Appendix	a Table	
1	Agglutination table	50
2	Summary table of antibody titer at different treatment	51
3	Summary table of red blood cells	52
4	Summary table of White blood cells	53

# IMMUNE RESPONSE OF NILE TILAPIA (Oreochromis niloticus) ON Aeromonas hydrohila ISOLATED FROM TAAL LAKE

#### JENIELYN VELASQUEZ PONIENTE

An undergraduate thesis submitted to the faculty of Biological Science
Department, College of Arts and Sciences, Cavite State University, Indang Cavite i
partial fulfillment of the requirements for the degree of Bachelor of Science in Biolog
major in Microbiology with Contribution No Prepared under the
supervision of Mr. Francisco Heralde III and Dr. Yolanda A. Ilagan.

#### INTRODUCTION

Aquaculture, as a sector of fish industry, has become a growing protein source of populace in the coming decade as sea-caught fish volumes decline from overlapping and illegal activities (Yap, 1998). It is important to the developing countries like the Philippines to have a well- developed aquaculture industry as other sources of protein are not always at low cost (Timm, 1998).

According to the food and nutrition survey by the Food and Nutrition Research Institute, the per capita consumption of fish in 1987 was estimated at 40 kilograms, of which 15 kilograms are fresh fish.

In 1996, a 44.4 percent increase from 1989 fish volume of over 7.110 million metric tons was recorded. The fish industry contributed 5.7 percent of the gross national product in the same period (Phil. Fisheries Profile, 1997).

The population of the Philippines in 1996 was 68 million (National Statistics Office) and following this rate of growth, the population is expected to reach 75 million