

IMMUNE RESPONSE TO STRESS INDUCED BY CORTICOSTEROID
IN COMMON CARP (*CYPRINUS CARPIO* LINNAEUS)
EXPOSED TO *AEROMONAS HYDROPHILA*

SUSAN C. LUMANLAN

MASTER OF SCIENCE
UNIVERSITI PERTANIAN MALAYSIA

1993

TW 2102

1000383184

25303

tesis
SH 167 .C3 L8 1993



1000383184
Immune response to stress induced by corticosteroid in commu
carp (cyprinus carpio linnaeus) exposed to aeromonas
hydrophila / Susan C.Lumanlan.



PERPUSTAKAAN SULTANAH NUR ZAHIRAH
UNIVERSITI MALAYSIA TERENGGANU (UMT)
21030 KUALA TERENGGANU

CT. 1996

S
02

Lihat sebelah

SH
167
C3
L8
1993

HAK MILIK
PERPUSTAKAAN KUSTEM

RESPONSE TO STRESS INDUCED BY CORTICOSTEROID
IN COMMON CARP (*CYPRINUS CARPIO* LINNAEUS)
EXPOSED TO *AEROMONAS HYDROPHILA*

Partial Fulfillment of the Requirements for the
Degree of Science in the Faculty of Fisheries
Universiti Pertanian Malaysia

1000383184

ACKNOWLEDGEMENTS

**IMMUNE RESPONSE TO STRESS INDUCED BY CORTICOSTEROID
IN COMMON CARP (*CYPRINUS CARPIO LINNAEUS*)
EXPOSED TO *AEROMONAS HYDROPHILA***

My sincere appreciation and thanks to my committee members, Dr. Sharr Anni Harmin, for his wise counsel, motivation and support and to Prof. N.S. Yadav, from the Department of Genetics and Cellular Biology, University Malaya, for his significant and helpful comments and suggestions throughout this study.

To Dr. Gary Nash, I express my gratitude for his assistance in the interpretation of the histological materials. My heartfelt thanks to Mr. Hadi Mariani for his help with the **SUSAN C. LUMANLAN**, to Dr. Rosnida, Faculty of Veterinary Medicine and Animal Science, UPM, for generously providing fresh sheep blood used in this study and to Mr. Anuar Basir of Koko Malaysia, Sdn. Bhd. who kindly provided the cocoa butter used in the experiments.

I am also thankful to the Faculty of Fisheries and Marine Science staff particularly, Rosli Aslan, Abdul Ghani, Abdullah Jaafar, Yuzaini Ahmad, Suriani Sidek and Sairina for their assistance in various ways to accomplish this study.

Thesis Submitted in Fulfilment of the Requirements for the
Degree of Master of Science in the Faculty of Fisheries
and Marine Science, Universiti Pertanian Malaysia

January 1993

1000383184

5
7
C3
8
1993

ACKNOWLEDGEMENTS

I am immensely grateful to my chairman Dr. Hassan Hj. Mohd. Daud, for his valuable advice, untiring guidance and encouragement. My sincere appreciation and thanks to my committee members, Dr. Sharr Azni Harmin, for his wise counsel, motivation and support and to Prof. M.S. Yadav, from the Department of Genetics and Cellular Biology, University Malaya, for his significant and helpful comments and suggestions throughout this study.

To Dr. Gary Nash, I express my gratitude for his assistance in the interpretation of the histological materials. My heartfelt thanks to Mr. Hadi Surianto for his help with the statistical analysis, to Dr. Roshida, Faculty of Veterinary Medicine and Animal Science, UPM, for generously providing fresh sheep blood used in this study and to Mr. Anuar Kasim of Koko Malaysia, Sdn. Bhd. who kindly provided the cocoa butter used in the experiments.

I am also thankful to the Faculty of Fisheries and Marine Science staff particularly, Rosli Aslim, Abdul Ghani, Abdulah Jaafar, Yusaini Ahmad, Suriani Sidek and Zairina Raden Zainal for their generous help in different ways to accomplish this study.

TABLE OF CONTENTS

My appreciation goes to the International Development Research Centre (IDRC) of Canada for providing the fellowship and research funding for this project.

LIST OF FIGURES

Special thanks is due to Dr. Juan D. Albaladejo, my colleague and friend who had patiently supported and assisted me throughout the duration of my study. I treasure the kindness and concern of Mr. and Mrs. Paul L. Manalo and their children, during my stay in Malaysia. I also cherish the friendship and cooperation of fellow students and friends in UPM and BFAR.

II REVIEW OF RELATED LITERATURE

This study is dedicated to a very special person, Alex M. Mayo, and my family, for their love, support and faith in me. Most of all, I give back all Praise and Glory to God, who had made all this possible.

Stress and the Immune System 15

Immunosuppression and Disease
Susceptibility in Fish 21

III EXPERIMENTAL PROCEDURES AND PRELIMINARY STUDIES 25

General Materials and Methods 26

Maintenance of Fish 28

Culture and Maintenance
of *Aeromonas hydrophila* 29

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	viii
LIST OF FIGURES	xi
LIST OF PLATES	xii
LIST OF FISH SPECIES	xv
ABSTRACT	xvii
ABSTRAK	xx
CHAPTER	
I GENERAL INTRODUCTION	1
II REVIEW OF RELATED LITERATURE	7
Concept of Stress	7
Stress Factors Affecting Fish	8
Physiological Response of Fish to Stress	11
Stress and the Immune System	16
Immunosuppression and Disease Susceptibility in Fish	21
III EXPERIMENTAL PROCEDURES AND PRELIMINARY STUDIES	28
General Materials and Methods	28
Maintenance of Fish	28
Culture and Maintenance of <i>Aeromonas hydrophila</i>	29

	Preparation of Bacterial and Cortisol Suspension	29
	Standard Curve of <i>Aeromonas</i> <i>hydrophila</i> Concentration	30
	Blood Glucose Assay	31
	Statistical Analysis	31
	Preliminary Experiments on Bacteria and Steroid Dosage	32
	Result of Median Lethal Dose (LD ₅₀) of <i>Aeromonas hydrophila</i>	33
	Result of Steroid Dose Response ...	35
IV	DETERMINATION OF THE EFFECT OF CORTISOL-INDUCED STRESS ON THE IMMUNE RESPONSE OF <i>CYPRINUS CARPIO</i> L. BY INDIRECT HAEMAGGLUTINATION TEST	
	Introduction	40
	Materials and Methods	42
	Preparation of Antigen for Haemagglutination	43
	Washing and Adsorption of Sheep Red Blood Cells (Srbc) with Antigen, <i>Aeromonas hydrophila</i>	43
	Determination of Antibody Titre ...	44
	Results	45
	Discussion	47
V	DETERMINATION OF THE EFFECT OF CORTISOL-INDUCED STRESS ON THE IMMUNE RESPONSE OF <i>CYPRINUS CARPIO</i> L. BY PASSIVE HAEMOLYTIC PLAQUE ASSAY	
	Introduction	55
	Materials and Methods	58

	Production of Plaque Assay Slides	59
	Preparation of Lymphocyte Suspension	59
	Preparation of Indicator Cells	60
	Adsorption of Sheep Red Blood Cells with Antigen	61
	Complement	61
	Plaquing Procedures	62
	Results	64
	Discussion	69
VI	HISTOPATHOLOGICAL INVESTIGATION OF HAEMOPOIETIC TISSUES OF <i>CYPRINUS CARPIO</i> L. TO CORTISOL-INDUCED STRESS	78
	Introduction	80
	Materials and Methods	80
	Results	81
	Discussion	95
VII	CHANGES IN BLOOD GLUCOSE LEVELS IN FOLLOWING <i>CYPRINUS CARPIO</i> L. CORTISOL-INDUCED STRESS	
	Introduction	100
	Materials and Methods	102
	Results	102
	Indirect Haemagglutination Study ...	102
	Haemolytic Plaque Study	106

	Histopathology Study	109
Table	Discussion	109
	VIII GENERAL DISCUSSION, CONCLUSION AND SUGGESTIONS FOR FUTURE STUDIES	119
	REFERENCES	129
	APPENDIX	151
	BIOGRAPHICAL SKETCH	163
	Lymphoid Cells in <i>C. carpio</i> Inoculated with <i>A. hydrophila</i> 24 Hrs After Implantation of Cortisol (10 µg/gm body wt)	66
4	Mean (±S.D.) Plaque Forming Cell Count in <i>C. carpio</i> of Five Treatment Groups	67
5	Mean Blood Glucose Levels (mg/100 ml) of <i>C. carpio</i> in Five Treatment Groups from Indirect Haemagglutination Study	104
6	Mean Blood Glucose Levels (mg/100 ml) of <i>C. carpio</i> in Five Treatment Groups from Haemolytic Plaque-Study	107
7	Mean Blood Glucose Levels (mg/100 ml) of <i>C. carpio</i> in Five Treatment Groups from Histopathology Study	110
8	Summary of Water Quality Parameters Taken in the Duration of the Experiment	151
9	Median Lethal Concentration (LD ₅₀) of <i>A.</i> <i>hydrophila</i> in <i>C. carpio</i> Analyzed Using the Spearman-Kärber Method (Hamilton et al., 1977)	151
10	Results of the Preliminary Standardization Techniques of Antigen (<i>A. hydrophila</i>) Coated Sheep Red Blood Cells (SRBC) for Determination of Optimal Antibody Titre for Indirect Haemagglutination Test	152

LIST OF TABLES

Table		Page
1	LD ₅₀ of <i>Aeromonas hydrophila</i> on Common Carp (<i>Cyprinus carpio</i> L.)	34
2	Serum Antibody Titre of <i>C. carpio</i> Inoculated with <i>A. hydrophila</i> and Cortisol+ <i>A. hydrophila</i>	46
3	Number of Plaque Forming Cells (PFC)/10 ⁵ Lymphoid Cells in <i>C. carpio</i> Inoculated with <i>A. hydrophila</i> 24 Hrs After Implantation of Cortisol (10 µg/gm body wt)	66
4	Mean (±S.D.) Plaque Forming Cell Count in <i>C. carpio</i> of Five Treatment Groups	67
5	Mean Blood Glucose Levels (mg/100 ml) of <i>C. carpio</i> in Five Treatment Groups from Indirect Haemagglutination Study	104
6	Mean Blood Glucose Levels (mg/100 ml) of <i>C. carpio</i> in Five Treatment Groups from Haemolytic Plaque Study	107
7	Mean Blood Glucose Levels (mg/100 ml) of <i>C. carpio</i> in Five Treatment Groups from Histopathology Study	110
8	Summary of Water Quality Parameters Taken in the Duration of the Experiment'	151
9	Median Lethal Concentration (LD ₅₀) of <i>A. hydrophila</i> in <i>C. carpio</i> Analyzed Using the Spearman-Karber Method (Hamilton et al., 1977)	151
10	Results of the Preliminary Standardization Techniques of Antigen (<i>A. hydrophila</i>) Coated Sheep Red Blood Cells (Srbc) for Determination of Optimal Antibody Titre for Indirect Haemagglutination Test	152

11	Exclusion Test of Viable and Dead Lymphoid Cells from Spleen and Kidney Tissues in <i>C. carpio</i>	152
12	Differential Cell Count in Kidney Smears of <i>C. carpio</i> Inoculated with <i>A. hydrophila</i>	153
13	Summary of Plaque Forming Cells Count of Sensitized Lymphoid Cells Layered with Bacteria - Coated Srbc Incubated in Different Complement Preparations	154
14	Multiple Range Test of Cortisol Treatment of 10 µg/gm body wt at Pre and Post Administration in <i>C. carpio</i>	155
15	Multiple Range Test of PFC at Week 1 Between Treatment Groups in <i>C. carpio</i>	155
16	Multiple Range Test of PFC at Week 2 Between Treatment Groups in <i>C. carpio</i>	155
17	Multiple Range Test of PFC at Week 3 Between Treatment Groups in <i>C. carpio</i>	156
18	Multiple Range Test for Blood Glucose Assay of Cocoa Butter Treatment in <i>C. carpio</i> for Five Sampling Days from Indirect Haemagglutination Study	156
19	Multiple Range Test for Blood Glucose Assay of Cortisol Treatment in <i>C. carpio</i> for Five Sampling Days from Indirect Haemagglutination Study	156
20	Multiple Range Test for Blood Glucose Assay of Bacteria Treatment in <i>C. carpio</i> for Five Sampling Days from Indirect Haemagglutination Study	157
21	Multiple Range Test for Blood Glucose Assay Between Treatment Groups in <i>C. carpio</i> at Day 14 Post Injection from Indirect Haemagglutination Study	157

22	Multiple Range Test for Blood Glucose Assay of Cocoa Butter Treatment in <i>C. carpio</i> for Four Weeks Sampling Period from Haemolytic Plaque Study	157
23	Multiple Range Test of Blood Glucose Assay in <i>C. carpio</i> Between Treatment Groups at Day 28 Post Injection from Histopathology Study	158
24	ANOVA for Steroid Dose Response of Pre and Post Administration of 10 µg/gm body wt Cortisol in <i>C. carpio</i>	158
25	ANOVA for PFC at Week 1 Post Injection Between Treatment Groups in <i>C. carpio</i>	158
26	ANOVA for PFC at Week 2 Post Injection Between Treatment Groups in <i>C. carpio</i>	159
27	ANOVA for PFC at Week 3 Post Injection Between Treatment Groups in <i>C. carpio</i>	159
28	ANOVA for Blood Glucose Assay of Cocoa Butter Treatment in <i>C. carpio</i> for Five Sampling Days from Indirect Haemagglutination Study	159
29	ANOVA for Blood Glucose Assay of Cortisol Treatment in <i>C. carpio</i> for Five Sampling Days from Haemagglutination Study	160
30	ANOVA for Blood Glucose Assay of Bacteria Treatment in <i>C. carpio</i> for Five Sampling Days from Haemagglutination Study	160
31	ANOVA for Blood Glucose Assay Between Treatment Groups in <i>C. carpio</i> at Day 14 Post Injection Haemagglutination Study	160
32	ANOVA for Blood Glucose Assay of Cocoa Butter Treatment in <i>C. carpio</i> For Four Weeks Sampling Period from Haemolytic Plaque Assay Study	161
33	ANOVA for Blood Glucose Assay Between Treatment Groups in <i>C. carpio</i> at Day 28 Post Injection from Histopathology Study	161

LIST OF FIGURES

Figure	Description	Page
1	Mean Blood Glucose Levels for Different Dosages of Cortisol Preparations in <i>C. carpio</i> from Pre and Post Treatment Period	39
2	Serum Haemagglutinating Antibody Titres from 40 Pooled Samples of <i>C. carpio</i> Injected Cortisol + <i>A. hydrophila</i> and <i>A. hydrophila</i> Alone	48
3	Serum Haemagglutinating Antibody Titre of Treated <i>C. carpio</i> injected with Cortisol + <i>A. hydrophila</i> and <i>A. hydrophila</i> Alone	49
4	Plaque Forming Cell Counts in <i>C. carpio</i> Treated with Cortisol and Inoculated with <i>A. hydrophila</i>	68
5	Mean Blood Glucose Levels of <i>C. carpio</i> from Five Treatment Groups from Indirect Haemagglutination Study	105
6	Mean Blood Glucose Levels of <i>C. carpio</i> from Five Treatment Groups from Haemolytic Plaque Study	108
7	Mean Blood Glucose Levels of <i>C. carpio</i> from Five Treatment Groups in Histopathology Study	111
8	Standard Curve of <i>Aeromonas hydrophila</i> Concentration Against Optical Density Read at 600 nm	162
9	Thymic section of <i>C. carpio</i> with Cortisol at 28 dpi	82
10	Clusters of Macrophages in Thymic Tissue of Cortisol and Bacteria Inoculated <i>C. carpio</i> at 7 dpi	84

LIST OF PLATES

Plates		Page
1	Deep Ulcer Formation Characterized by Putrefactive Necrosis into the Underlying Muscles of <i>C. carpio</i> Inoculated with 10^4 Cells/ml <i>A. hydrophila</i> After 72 Hours	36
2	Tissue Section of the Necrotic Muscle Showing Moth-Eaten Appearance and Mononuclear Inflammatory Cells Invasion	36
3	Muscle Fibers Undergoing Liquefaction Necrosis Seen After Inoculation of 10^4 cells/ml of <i>A. hydrophila</i> after 2 dpi	37
4	Higher Magnification of Necrotic Muscle Fiber Showing Polymorphonuclear and Mononuclear Inflammatory Cells Infiltration	37
5	Lymphoid Cells from Control Fish " Suspended with Sheep Red Blood Cells (Srbc) Showing Absence of Plaque	63
6	Plaque Formation Caused by Sensitized Lymphoid Cells from <i>C. carpio</i> Inoculated with <i>A. hydrophila</i> . Antibody Forming Cell Located in the Center of the Plaque Surrounded by Halo Area Developed by Lysed Srbc	63
7	Photomicrographs of Haemopoietic Cells from Kidney Smear Preparation: Lymphocyte, Macrophage, Agranuloblast Granuloblast	65
8	Thymic Tissue Showing Vacuolation and Hydropic Degeneration of Thymocytes in <i>C. carpio</i> with Cortisol at 14 dpi	82
9	Thymic Section of <i>C. carpio</i> with Cortisol at 28 dpi	82
10	Clusters of Macrophage in Thymic Tissue of Cortisol and Bacteria Inoculated <i>C. carpio</i> at 7 dpi	84

11	Hassal's Corpuscles-like Structures in Thymus from Cortisol Injected <i>C. carpio</i> at 14 dpi.	84
12	Thymus of <i>C. carpio</i> Sampled at 28 dpi	85
13	Normal Thymic Tissue of Saline Injected <i>C. carpio</i> Showing Basophilic Staining Property	85
14	Hydropic Degeneration Seen in Interstitial Tissue of <i>C. carpio</i> 's Spleen Treated with Cortisol+Bacteria at 21 dpi	87
15	Focal Necrosis Observed in Spleen of Bacteria Inoculated <i>C. carpio</i> at 21 dpi	87
16	Higher Magnification of Focal Necrosis Showing Presence of Bacteria	88
17	Enlarged Melanomacrophage Centre in the Spleen of Bacteria Inoculated <i>C. carpio</i> at 21 dpi	88
18	Severe Congestion of the Splenic and Pancreatic Vessels in Bacteria Inoculated Group at 21 dpi	90
19	Haemopoietic Tissue of Kidney from Cortisol+Bacteria Inoculated <i>C. carpio</i> at 21 dpi Showing Cell Necrosis, Vesiculation of the Nucleus and Hydropic Degeneration	90
20	Section of Haemopoietic Tissue of Kidney Showing Extensive Vacuolation in Cortisol+Bacteria Inoculated <i>C. carpio</i> at 28 dpi	91
21	Kidney Tubular Cells of Cortisol Injected <i>C. carpio</i> Sampled at 21 dpi Undergoing Necrosis of the Proximal Tubules	91
22	Bowman's Capsule of Cortisol+Bacteria Inoculated Group at 21 dpi Exhibiting Accumulation of Fluid in Intercapsular Space	93

23	Kidney Interstitial Tissue Showing Proliferation of Macrophage Seen in Bacteria Inoculated <i>C. carpio</i> at 7 dpi	93
24	Squash Smear of Head Kidney in <i>C. carpio</i> at 7 dpi Showing Macrophage with Bacteria in the Cytoplasm	94

Bluegill	<i>Lepomis macrochirus</i>
Blue gourami	<i>Trichogaster trichopterus</i>
Bream	<i>Abramis brama</i>
Brock charr trout	<i>Salvelinus fontinalis</i>
Brown bullhead	<i>Ictalurus nebulosus</i>
Brown trout	<i>Salmo trutta</i>
Channel catfish	<i>Ictalurus punctatus</i>
Char	<i>Salvelinus</i> sp.
Chinook salmon	<i>Oncorhynchus tshawytscha</i>
Coho salmon	<i>Oncorhynchus kisutch</i>
Common carp	<i>Cyprinus carpio</i>
Cunner	<i>Tautoglabrus adspersus</i>
Cutthroat trout	<i>Oncorhynchus clarki</i>
Desert pupfish	<i>Cyprinodon n. nevadensis</i>
Eel	<i>Anguilla anguilla</i>
Featherback	<i>Notopterus notopterus</i>
Goldfish	<i>Carassius auratus</i>
hog choker	<i>Trisectes marisus</i>
Johnny darter	<i>Etheostoma nigrum</i>
Killifish	<i>Fundulus heteroclitus</i>

LIST OF FISH SPECIES

American shad	<i>Alosa sapidissima</i>
Atlantic salmon	<i>Salmo salar</i>
Bluegill	<i>Lepomis macrochirus</i>
Blue gourami	<i>Trichogaster trichopterus</i>
Bream	<i>Abramis brama</i>
Brook charr trout	<i>Salvenilus fontinalis</i>
Brown bullhead	<i>Ictalurus nebulosus</i>
Brown trout	<i>Salmo trutta</i>
Channel catfish	<i>Ictalurus punctatus</i>
Char	<i>Salvenilus spp.</i>
Chinook salmon	<i>Oncorhynchus tshawytscha</i>
Coho salmon	<i>Oncorhynchus kisutch</i>
Common carp	<i>Cyprinus carpio</i>
Cunner	<i>Tautogolabrus adspersus</i>
Cutthroat trout.....	<i>Oncorhynchus clarki</i>
Desert pupfish	<i>Cyprinodon n. nevadensis</i>
Eel	<i>Anguilla anguilla</i>
Featherback	<i>Notopterus notopterus</i>
Goldfish	<i>Carassius auratus</i>
Hog chocker	<i>Trinectes marlatus</i>
Johnny darter	<i>Etheostoma nigrum</i>
Killifish	<i>Fundulus heteroclitus</i>

Abstract of the thesis submitted to the Senate of the
 Universiti Pertanian Malaysia in fulfillment of the

Killifish	<i>Fundulus heteroclitus</i>
Largemouth bass	<i>Micropterus salmoides</i>
Medaka	<i>Oryzias latipes</i>
Mossambique mouthbrooder	<i>Tilapia mossambica</i>
Mudfish	<i>Labeo umbratus</i>
Mudsucker	<i>Labeo capensis</i>
North American Eel	<i>Anguilla rostrata</i>
Northern pike	<i>Esox lucius</i>
Perch	<i>Perca fluviatilis</i>
Plaice	<i>Pleuronectes platessa</i>
Rainbow trout	<i>Oncorhynchus mykiss</i>
River carp	<i>Puntius schwanenfeldii</i>
Sea/striped bass	<i>Morone saxatilis</i>
Sea mullet	<i>Mugil cephalus</i>
Silver carp	<i>Hypophthalmichthys molitrix</i>
Sockeye salmon	<i>Oncorhynchus nerka</i>
Spot	<i>Leiostomus xanthurus</i>
Thread fin shad	<i>Dorosoma petenense</i>
Tilapia	<i>Sarotherodon aureus</i>
White sucker	<i>Catostomus commersoni</i>

The marked reduction in haemolytic plaque forming
 cells in cortisol treated carps indicated the suppression
 of antibody mediated response. Similarly, a quantitative

Abstract of the thesis submitted to the Senate of the
Universiti Pertanian Malaysia in fulfilment of the
requirement for the degree of Master of Science

**IMMUNE RESPONSE TO STRESS INDUCED BY CORTICOSTEROID
IN COMMON CARP (*CYPRINUS CARPIO LINNAEUS*)
EXPOSED TO *AEROMONAS HYDROPHILA***

By

Susan C. Lumanlan

January 1993

Chairman : Dr. Hassan Hj. Mohd. Daud

Faculty : Fisheries and Marine Science

The effect of simulated stress induced by cortisol implantation in common carp (*Cyprinus carpio*) was studied. Humoral mediated responses to injected antigens following cortisol treatment were assessed by using the passive haemolytic plaque technique and indirect haemagglutination assay. A single cortisol implant and subsequent challenge to *Aeromonas hydrophila* elicited modulation in the fish immune system and also histopathological changes in the kidney, spleen and thymus. Hyperglycaemia which is a stress related physiological change was associatively manifested.

The marked reduction in haemolytic plaque forming cells in cortisol treated carps indicated the suppression of antibody mediated response. Similarly, a quantitative

decrease in haemagglutinating antibody titre suggested that the immunosuppressive action of cortisol could have a pernicious effect on the fish's ability to resist infection. The repression of the ability of the lymphoid cells i.e haemolytic plaque forming cells from the kidney and spleen to secrete antibodies against *A. hydrophila* was demonstrated. It appeared that the cortisol effect was due to the suppression of the differentiation and maturation of antibody forming precursor cells in the tissues studied.

Histopathological studies of the lymphoid organs showed pronounced vacuolation and hydropic degeneration in thymus, kidney and spleen. These marked changes correlated with the functional parameters noted in the immune response. Blood glucose levels which were used as indicator of stress condition, fluctuated in weekly samples in all groups, but in general showed a pattern of initial low levels followed by elevated level in the cortisol treated fish.

The results of this study indicated that the ability of fish to mount an immune response was decreased in the presence of cortisol-mediated stress. While sublethal stress did not detrimentally manifest in the development

of clinical disease infections as a direct effect, it nevertheless was considered an important element in limiting aquaculture production by reducing the optimum immune functions of fish.