



UNIVERSITI PUTRA MALAYSIA

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

SUSAN C. LUMANLAN

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EXPOSED TO *AEROMONAS HYDROPHILA***

By

SUSAN C. LUMANLAN

Thesis Submitted in Fulfilment of the Requirements for the
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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
Discussion	109
VIII GENERAL DISCUSSION, CONCLUSION AND SUGGESTIONS FOR FUTURE STUDIES	119
REFERENCES	129
APPENDIX	151
BIOGRAPHICAL SKETCH	163



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 <i>et al.</i> , 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		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



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



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>Salvelinus fontinalis</i>
Brown bullhead	<i>Ictalurus nebulosus</i>
Brown trout	<i>Salmo trutta</i>
Channel catfish	<i>Ictalurus punctatus</i>
Char	<i>Salvelinus spp.</i>
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 chocker	<i>Trinectes marlatus</i>
Johnny darter	<i>Etheostoma nigrum</i>
Killifish	<i>Fundulus heteroclitus</i>



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>



Abstract of the thesis submitted to the Senate of the
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By

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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.



Abstrak tesis yang diserahkan kepada Senat Universiti
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memenuhi keperluan ijazah Master Sains

**RESPONS IMUN TERHADAP STRES YANG DIARUH
OLEH KORTIKOSTERIOD DALAM IKAN LEE KOH
(*CYPRINUS CARPIO LINNAEUS*) DIDEDAHKAN
KEPADA *AEROMONAS HYDROPHILA***

oleh

Susan C. Lumanlan

Januari 1993

Pengurus : Dr. Hassan Hj. Mohd. Daud

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Kesan stres simulasi yang diaruh dengan cara pengimplanan kortisol dalam ikan Lee Koh (*Cyprinus carpio*) telah dikaji. Respons keimunan humoral terhadap suntikan antigen selepas rawatan kortisol dinilai melalui teknik plak hemolitik pasif dan hemaglutinasi tidak langsung. Satu implantasi tunggal kortisol dan diikuti dengan cabaran dengan *Aeromonas hydrophila* menunjukkan perubahan dalam sistem imun ikan dan juga histopatologi ginjal, limfa dan timus. Hiperglisemia iaitu perubahan fisiologi berkait dengan stres juga ditunjukkan.

Pengurangan nyata jumlah pembentukan plak hemolitik dalam ikan kap yang dirawat dengan kortisol menandakan terdapat tekanan terhadap pengeluaran antibodi. Begitu



juga, penurunan kuantitatif dalam titer antibodi hemaglutinan menunjukkan bahawa tindakan imunosupresif kortisol akan memberikan kesan merbahaya terhadap kemampuan ikan untuk melawan jangkitan. Penekanan terhadap kemampuan sel-sel limfoid iaitu sel pembentuk plak hemolitik yang berasal dari ginjal dan limfa untuk merembeskan antibodi melawan *A. hydrophila* telah ditunjukkan. Kesan itu dianggap disebabkan oleh perencatan sel-sel prekursor pembentuk antibodi dalam tisu-tisu tersebut.

Kajian histopatologi menunjukkan perubahan dalam organ-organ limfoid termasuk vakuolasi dan degenerasi hidropik yang ketara dalam timus, ginjal dan limfa. Perubahan-perubahan ini selari dengan parameter-parameter yang dilihat dalam respons imun. Glukosa darah sebagai petanda keadaan stres, walaupun turun naik dalam sampel-sampel mingguan dalam semua kumpulan, menunjukkan pola am di mana nilai permulaan yang rendah tetapi meningkat kepada aras yang lebih tinggi di dalam ikan yang dirawat dengan kortisol.

Keputusan kajian ini menunjukkan bahawa kemampuan ikan untuk memberikan respons keimunan humoral adalah menurun dalam kehadiran keadaan stres aruhan kortisol.

Walaupun stres pada aras subletal tidak menonjolkan tanda-tanda klinikal sebagai efek langsung, ianya walau bagaimanapun disifatkan sebagai elemen yang penting dalam pengeluaran akuakultur dengan mengurangi fungsi optimum keimunan ikan.



CHAPTER I

GENERAL INTRODUCTION

Stress in general terms, reflects a state of decrease in fitness induced by an external agent or "stressor" which alters the homeostatic mechanisms of the organism and threatens its survival (Colombo et al., 1990). Stress may be induced by deleterious change in the environment which then causes a disturbance of the normal homeostatic mechanisms within the fish (Pickering, 1981).

Changes in the physiological system involves the defense system which are comprised of various responses including tissue repair, phagocytosis, inflammation and numerous specific and non-specific responses mediated by the lymphoid system.

Like all vertebrates, fish possess a wide array of defense systems to protect themselves against infections with disease causing organisms. Under favourable conditions, these systems control the pathogen-loading of fish to such an extent that disease or impairment of the normal physiological function of the body is prevented. However, under conditions of stress, the defense system



can breakdown and disease may be caused by organisms that are under normal conditions relatively harmless.

Ellis (1981) noted that stress can cause a derangement of the defense system with subsequent suppression or exaggeration of immune responses. These in turn disturbed the balance of other physiological systems and subsequently resulted in disease. Modulation of the defense system during stress could either increase or decrease the response of fish to the infection.

There is still much to be learned of the relationship of stress response and the relative susceptibility of fish to infection. Interaction of stress factors modulating the defense system and its relation to disease is extremely complex and barely understood. Researches have been made steadily through the years to assess the over-all concept of this interaction. Wedemeyer (1970) and Snieszko (1974) have established that stress can lower the resistance of fish to disease which could trigger an outbreak of infectious disease in fish population.

One component of the physiological response of the fish to stress is the stimulation of hypothalamic-pituitary-interrenal (HPI) axis resulting in the increase of circulating corticosteroids (Donaldson, 1981). Prolonged elevation of blood cortisol levels during chronic stress can predispose the fish to disease by suppressing the defense system (Pickering, 1981; 1989a).

