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

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By

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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

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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

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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 Pertanian Malaysia sebagai syarat bagi 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

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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 prekusor 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).