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THE EFFECT OF IMMUNE ENHANCER ON THE NON-SPECIFIC DEFENSE MECHANISM OF RED TILAPIA HYBRID *(OREOCHROMIS NILOTICUS X OREOCHROMIS MOSSAMBICUS*) CHALLENGED WITH *AEROMONAS HYDROPHILLA*

JOSELITO R. SOMGA

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BY

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LIST OF ABBREVIATIONS

CFU	Colony Forming Unit
CP	Crude Protein
DMSO	Dimethyl sulfoxide
EDTA	Ethylene Diamine Tetraacetic Acid
EUS	Epizootic Ulcerative Syndrome
LD_{50}	Median Lethal Dose
MMC	Melanomacrophages Center
MS-222	Tricane Methanesulfonate
NBT	Nitroblue Tetrazolium
PBC	Post Bacterial Challenge
PBS	Physiological Buffered Saline
RBC	Red Blood Cells
RS	Reimlers Schott
SD	Standard Deviation
TSA	Trypticase Soy Agar
WBC	White Blood Cells

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

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Immunomodulation of ENCAP in red tilapia hybrid against *Aeromonas hydrophila* was studied. Different concentrations of ENCAP (0, 500, 750 and 1000 mg/kg of feed) were fed to different groups of fish and later challenged by intraperitoneal injection of 8×10^8 CFU/ml *A. hydrophila*. The non-specific immune response was determined after one, two, four and seven days post bacterial challenge using haematological and serological assays such as haematocrit, WBC counts, potential killing activity of neutrophils and other phagocytic cells by NBT, lysozyme activity and total plasma protein. Different concentrations of ENCAP showed different levels of immunopotentiation.

Hematocrit levels and WBC counts decreased in all the groups due to migration of erythrocytes and leukocytes to the infected areas. Probably, toxins released by the bacteria also contributed to these lowered levels. Neutrophils and other phagocytic cells demonstrated an increase in the potential killing activity. Lysozyme activity also increased in fish fed with ENCAP, while total plasma protein decreased brought about by the abnormal function of the liver to synthesize protein. Based on these cellular and humoral factors, fish fed with 750 mg/kg ENCAP had a consistently higher immune response. Fish fed with 500 mg/kg and 1000 mg/kg showed a lower immune response which suggests slight immunopotentiation and mild immunosuppression, respectively.

Histopathology showed that both the control and fish fed with different concentrations of ENCAP exhibited varying lesions in the spleen, liver, pancreatic tissue and kidney. However fish fed with ENCAP showed a significantly higher survivability. Results of this study indicated that ENCAP caused immunomodulation. The enhanced non-specific response contributed to the increased survivability.

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KESAN PERANGSANG IMMUN KE ATAS MEKANISME PERTAHANAN TIDAK SPECIFIK DALAM HIBRID TILAPIA MERAH (*OREOCHROMIS NILOTICUS* X *OREOCHROMIS MOSSAMBICUS*) YANG DICABAR DENGAN *AEROMONAS HYDROPHILA*

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Immunomodulasi oleh ENCAP pada hibrid tilapia merah terhadap *Aeromonas hydrophila* telah dikaji. Kepekatan ENCAP yang berbeza (0, 500, 750 dan 1000 mg/kg makanan) telah diberi kepada beberapa kumpulan ikan dan kemudian dicabar dengan suntikan intraperitoneal 8 x 10^8 CFU/ml *A. hydrophila*. Tindakbalas tidak specifik imun telah ditentukan pada hari pertama, kedua, keempat dan ketujuh selepas suntikan bakteria dengan menggunakan hematologikal dan serologikal asei seperti hematokrit, pengiraan sel darah putih, potensi aktiviti membunuh neutrofil dan sel-sel fagositik lain secara NBT, aktiviti lisozim dan jumlah protein plasma. Kepekatan ENCAP yang berbeza menunjukkan tahap immunopotensasi yang berbeza.

Paras hematokrit dan jumlah WBC berkurangan dalam semua kumpulan disebabkan oleh migrasi eritosit dan leukosit ke kawasan yang dijangkiti. Mungkin, toksin yang dilepaskan oleh bakteria menyumbang kepada tahap yang rendah tersebut. Neutrofil dan sel-sel fagositik lain menunjukkan peningkatan potensi aktiviti membunuh. Aktiviti lisozim telah juga ditingkatkan pada ikan yang diberi makan ENCAP. Sementara jumlah protein plasma berkurangan akibat dari fungsi abnormal hati mengsintesiskan protein. Berdasarkan faktor-faktor sellular dan humoral, ikan yang diberi makan 750 mg/kg ENCAP mempunyai tindakbalas immunisasi tinggi yang berpanjangan. Ikan yang diberi makan 500 mg/kg menunjukkan tindakbalas immunisasi rendah yang mencadangkan sedikit immunopotensasi dan manakala pada 1000 mg/kg menunjukkan immunosupresi.

Histopatologi menunjukkan kedua-dua kawalan dan ikan yang diberi makan dengan kepekatan ENCAP yang berlainan mempamirkan lesi yang berbeza dalam limpa, hati, tisu pankreatik dan ginjal. Walaubagaimanapun, ikan yang diberi makan dengan ENCAP menunjukkan kemandirian yang jelas tinggi. Keputusan kajian ini menunjukkan bahawa ENCAP menyebabkan immunomodulasi. Tindakbalas tidak specifik yang diransang menyumbang kepada peningkatan kemandirian.

CHAPTER I

GENERAL INTRODUCTION

Aquaculture plays a vital role in the production of fish and other fishery products. Aquaculture has expanded around the world due to the increasing demand of protein from the growing human population and the decline of available natural aquatic resources. However, the rapid expansion and intensification of fish farming lead to the occurrence of various economically important diseases. Consideration on the intimate relationship between the fish, pathogen and environment seems to be neglected. The unwise increase of stocking density together with the deterioration of the aquatic environment can cause stress to the cultured fish. Stress lowers the resistance of the fish thereby giving chance to opportunistic pathogens to become invasive. Thus fish in this scenario, will inevitably succumb to diseases cause by either viruses, bacteria, parasites and fungi.

To overcome such problems, fish culturists became more dependent on the use of chemotherapeutic agents. But with the limitation of approved chemotherapeutic products, overused or misused of antibiotics generate the risk of bacterial resistant pathogens and the problems of drug residues in the environment and fish products (Ellis, 1988; Ghittino *et al.*, 1984; Anderson, 1992; Baticados and Paclibare, 1992; Nikl *et al.*, 1993). Rijkers *et al.* (1981) reported that prolonged used of oxytetracycline cause depression of the humoral and cellular immunity in common carp. Some chemicals such as malachite green, a known parasiticide and pyridylmercuric acetate, an effective fungicide cause cancer and mercury accumulation in tissues respectively (Anderson *et al.*, 1984).

The use of vaccine to stimulate the production of antibody against specific pathogen has been studied. The first experimental vaccination in fish was reported by Duff in 1942 against furunculosis using killed *Aeromonas salmonicida* given orally. But since then only few vaccines have been proven to be effective on commercial scale. Other vaccinations that have been successfully done experimentally were not reliably reproduced even using other techniques of administration and antigen preparation (Ellis, 1988). Although vaccination is a valuable approach for disease prevention (Alderman and Michel, 1991) its usefulness is limited by their specificity, lack of availability and high cost to produce commercially (Ellis, 1988; Yoshida *et al.*, 1993).

The constraints on the use of chemotherapeutic agents and vaccines in fish farming further the development of more effective ways and means to protect the fish from various disease causing organisms. The use and application of immunostimulants for protecting the fish against diseases has been attempted. Immunostimulant elevates the non-specific defense mechanism or the specific immune response (Anderson, 1992). This may be administered alone or in combination with vaccine to activate the non-specific defense mechanism as well as heightening the specific immune response.

The non-specific defense mechanism is the first line of defense which constitutes the protective barriers such as skin and scales, humoral factors in mucus and sera such as lysozymes, C-reactive protein, transferrin and interferon, and the cellular factors such as phagocytic cells, neutrophils and macrophages (Fletcher, 1986; Roberts, 1989; Robertsen *et al.*, 1990; Kaige *et al.*, 1990; Anderson, 1992). On the otherhand, the specific defense mechanism is responsible for initiating and mediating the humoral, cell mediated immunity (CMI) and the memory. The humoral immunity refers to the production of soluble antibody, whereas the CMI refers to responses which are mediated by lymphocytes and macrophages and the memory constitutes an adaptive change in the lymphoid cells causing an enhanced magnitude with subsequent challenge by the same antigen (Roberts, 1989).

The use of immunostimulants is being intensified in the areas of cancer and AIDS (Acquired immunodeficiency syndrome) research (Fudenberg and Whitten, 1984; Azuma and Jolles, 1987; WHO, 1990 as cited by Anderson, 1992). It activates macrophages, T- and B-lymphocytes, and natural killer cells that increase the body's ability to destroy tumour cells (Raa *et al.*, 1992). Immunostimulants were also used for activating early protection against diseases in domestic animals (Kehrli *et al.*, 1990).

Immunostimulants can be obtained from a very diverse natural sources and a large number have been made by chemical synthesis with natural products as structural models (Raa *et al.*, 1992). Different substances have been tested to stimulate immune response in fish. Glucans, a long-chain polysaccharides extracted from yeast given parenterally or orally were evaluated in fish for their ability to enhance protection against different bacterial pathogens (Yano *et al.*, 1989; Robertsen *et al.*, 1990; Raa *et al.*, 1992; Chen and Ainsworth, 1992; Nikl *et al.*, 1993; Jeney and Anderson, 1993).

Some drugs such as levamisole, quaternary ammonium compound (QAC) and short chain polypeptide (ISK) affect the non-specific defense mechanism activities (Jeney and Anderson, 1993). Immunoactive peptide *FK* 565 (Kitao and