



UNIVERSITI PUTRA MALAYSIA

**TOXICOLOGICAL AND IMMUNOLOGICAL EFFECTS OF DIAZINON ON
THE GREAT STURGEON (*HUSO HUSO*) OF NORTHERN IRAN**

HOSSEIN ALI KHOSHBAVAR ROSTAM

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BY

HOSSEIN ALI KHOSHBAVAR ROSTAMI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in the
Fulfilment of Requirements for the Degree of Doctor of Philosophy**

March 2008



DEDICATION

WITH LOVE AND APPRECIATION TO:

My parents: Mohamad Ali and Soghra Javan

My wife: Ommolbanin Kardar

My sons: Mohamad Ali and Mohsen

My daughter: Farzaneh

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Associate Professor Hassan Hj. Mohd. Daud, PhD

Faculty: Veterinary Medicine

Great sturgeon (*Huso huso*) is one of the highly valuable commercial fish species and the major economic resources of Northern Iran. Currently limited data are available on the immunophysiological responses of this valuable species. Unfortunately, within the last decade the average annual sturgeon harvest including great sturgeon from the South Caspian Sea has drastically reduced due to increase pollution problem. Diazinon is one of the most important organophosphorus pesticide groups commonly used in Iranian agriculture, including northern Iran in which both are the natural habitat and aquaculture sites of great sturgeon. In addition also, previous studies had shown that motile *Aeromonas* septicemia disease is one of the main factors in high mortality outbreaks in the sturgeon farming, particularly whenever the fish immune system is suppressed by some toxicants. Thus, the main objectives of this study were: (i) purification and characterization of great sturgeon immunoglobulin (IgM); (ii) determination of 96-hour LC₅₀ diazinon in great



sturgeon; (iii) assessment of some immunophysiological variables of fish following exposure to diazinon; and (iv) assessment of some immunoresponses of fish following treatment with some immunostimulators i.e. glucan and *Aeromonas hydrophila* antigen. In the immune response assessment, the IgM purified by affinity chromatography under non-reduced condition was found to be 870 kDa, as estimated by SDS-PAGE, while the MWt of the heavy and light chains under reduced condition were estimated at 77-84 and 28-30 kDa, respectively. In the diazinon toxicity study, the LC₅₀ value at 96-hour in fish weighing ca. 14 g under static water quality condition at 22°C was calculated as at 5.63 mg/L. Examination of haematological indices and tissue lysozyme of kidney, liver and spleen showed that exposure to diazinon at sublethal concentration of 1.5 mg/mL as long-term bath caused an effect similar to anaemia. Also, there were significant and insignificant changes in some blood parameters including immunocompetent cell populations, AST, ALP, ALT and LDH enzymes as well as chemiluminescence response of leucocytes at different days post-exposure to diazinon. An almost similar finding was observed when the glucan-injected fish (0.3 mg/kg bwt.) and fish immunized intraperitoneally with a single dose of formalin-killed *A. hydrophila* (5×10^7 cells/fish) were exposed to long-term, sublethal concentration of diazinon. However, some of these immunophysiological responses including respiratory burst, immunocompetent cell counts and lysozyme content were enhanced in both glucan-injected fish and immunized fish without diazinon bath. Also, the microagglutination antibody titer in immunized fish was higher than unimmunized fish.

In light microscope examinations there were congestion, haemorrhages, focal and generalized necrosis, cellular infiltration, hyperplasia and lamellae fusion as major

histopathological changes in the tissues of liver, kidney, spleen and gills of the fish exposed to sublethal dose of diazinon. Also, there were an increase in droplet materials blocking of club cell surfaces in nostrils and barbels tissues; a reduction in excretion of amorphous proteinaceous materials, increase in vesicle numbers and blockage of nostrils epithelial cell surfaces in diazinon exposed fish under scanning electron microscope examination.

In conclusion, short and long-term exposure of great sturgeon to diazinon at acute and chronic concentrations changes the basic blood cells constituents causing leucopenia, lymphopenia, neutrophilia, erythropenia, hyperglycemia, hypoproteinemia and decreased in both specific antibody production and leucocytes respiratory burst. Thus, avoiding the exposure of endangered juvenile fish to this pollutant is highly recommended.

Key words: great sturgeon, *Huso huso*, diazinon, glucan, *A. hydrophila*, lysozyme, chemiluminescence response, antibody, immunocompetent cells, immunoglobulin, LC₅₀ 96-hour.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KESAN DIAZINON TERHADAP TOKSIKOLOGIKAL
DAN IMUNOFISIOLOGIKAL DALAM STURGEON GERGASI, *HUSO*
HUSO (BRANDT, 1869)**

Oleh

HOSSEIN ALI KHOSHBAVAR ROSTAMI

Mac 2008

Pengerusi: Profesor Madya Hassan Hj. Mohd. Daud, PhD

Fakulti: Perubatan Veterinar

Sturgeon gergasi (*Huso huso*) adalah salah satu spesis ikan komersial bernilai tinggi dan menjadi sumber utama ekonomi di utara Iran. Pada masa ini, data mengenai tindakbalas imunofisiologi spesis yang bernilai ini amat terbatas. Walaubagaimana pun, dalam dekad kebelakangan ini, purata hasil tangkapan tahunan ikan sturgeon, termasuk sturgeon gergasi dari Laut Caspian Selatan telah menurun secara drastik kerana peningkatan masalah pencemaran. Diazinon merupakan salahsatu dari racun serangga penting kumpulan organofosfat yang digunakan secara meluas dalam pertanian Iran, termasuk di utara Iran yang mana merupakan habitat semulajadi dan tempat akuakultur sturgeon gergasi. Tambahan juga, kajian terdahulu telah menunjukkan bahawa penyakit septisemia *Aeromonas* motil adalah faktor utama dalam kejadian wabak kematian tinggi dalam penternakan sturgeon, terutamanya

apabila sistem imun ikan tertekan oleh setengah bahan toksik. Oleh itu objektif utama kajian ini adalah: (i) menyuling dan mencari imunoglobulin (IgM) sturgeon gergasi; (ii) menentu LC_{50} pada 96 jam diazinon dalam sturgeon gergasi; (iii) menilai beberapa pembolehubah imunofisiologi ikan selepas pendedahan terhadap diazinon; dan (iv) menilai beberapa tindakbalas imun ikan selepas rawatan dengan peransang imun iaitu glukukan dan antigen *Aeromonas hydrophila*. Dalam penilaian tindakbalas imun, IgM yang dituliskan dengan kromatografi keafinan dibawah keadaan tak berkurangan didapati seberat 870 kDa, sebagaimana dianggarkan dengan SDS-PAGE. Manakala berat molekul rantai berat dan rantai ringan adalah dianggarkan masing-masing pada 77-84 dan 28-30 kDa. Dalam ujian ketoksikan diazinon pada ikan seberat 14 g, nilai LC_{50} pada 96 jam, dalam keadaan air statik pada suhu 22°C adalah 5.63 mg/L. Pemeriksaan indek hematologikal dan lisozim tisu ginjal, hepar dan limfa menunjukkan bahawa pendedahan terhadap diazinon pada kepekatan subletal 1.5 mg/mL sebagai mandian jangka panjang menyebabkan kesan sama seperti anemia. Juga terdapat perubahan bererti dan tidak bererti dalam beberapa parameter darah termasuk populasi sel imunomampu, AST, ALP, ALT dan enzim LDH, dan juga tindakbalas kimipendarcahaya leukosit pada hari berbeza pasca pendedahan pada diazinon. Keputusan yang hampir sama dapat dilihat dalam ikan yang disuntik glukukan (0.3 mg/kg berat badan) dan ikan yang diimun dengan satu dos *A. hydrophila* yang dimati dengan formalin secara intraperitoneum (5×10^7 sel/ikan) apabila didedahkan secara jangka masa panjang pada kepekatan subletal diazinon. Walaubagaimana pun, beberapa tindakbalas imunofisiologikal termasuk letusan penafasan leukosit, bilangan sel imunomampu dan kandungan lisozim dipertingkatkan dalam ikan yang disuntik dengan glukukan dan yang diimun, tanpa

mandian diazinon. Didapati juga, mikroagglutinasi titer antibodi dalam ikan diimun adalah lebih tinggi dari ikan tidak diimun.

Dalam pemeriksaan mikroskop cahaya, perubahan histopatologi yang utama adalah kongesi, hemoraj, nekrosis fokal dan umum, infiltrasi sel dalam tisu hepar, ginjal, limfa dan hiperplasia dan penyantuman lamella dalam insang ikan yang terdedah pada dos subletal diazinon. Didapati juga, pemerhatian mikroskop elektron imbasan menunjukkan terdapat penambahan bahan titisan dan penutupan permukaan sel belantan dalam tisu nostril dan barbel, pengurangan dalam perkumuhan bahan protein amorfus, peningkatan jumlah vesikel dan penutupan permukaan sel epitelial nostril dalam ikan yang didedahkan pada diazinon.

Sebagai rumusan, pendedahan samaada secara jangka pendek atau panjang sturgeon gergasi pada diazinon pada kepekatan akut dan kronik mengubah konstitusi asas sel darah menyebabkan leukopenia, limfopenia, neutrofilia, eritropenia, hiperglisemia, hipoproteinemia dan pengurangan dalam pengeluaran antibodi spesifik dan letusan penafasan leukosit. Olehitu, mengelakkan pendedahan ikan juvenil yang terancam pada bahan pencemaran ini adalah amat disyorkan.

Perkataan kunci: sturgeon gergasi, *Huso huso*, diazinon, glukon, *A. hydrophila*, lisozim, tindakbalas kemopendafluor, antibodi, sel imunomampu, imunoglobulin, LC₅₀ pada 96 jam.

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I certify that an Examination Committee met on 14th March 2008 to conduct the final examination of HOSSEIN ALI KHOSHBAVAR ROSTAMI on his Doctor of Philosophy thesis entitled "Toxicological and immunophysiological effects of diazinon on the great sturgeon, *Huso huso* (Brandt)" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

Mohd. Hair Bejo, Ph.D.

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohamed Ali Rajion, Ph.D.

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Rasedee Abdullah, Ph.D.

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Benjamin, R. Mikryakov, Ph.D.

Professor
Institute for Biology of Inland Waters
Russian Academy of Sciences
(External Examiner)

HASANAH MOHD GAZALI, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Hassan Hj Mohd Daud, Ph.D.

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mehdi Soltani, Ph.D.

Professor
Faculty of Veterinary Medicine
University of Tehran
(Member)

Abdul Rahman Omar, Ph.D.

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

**HOSSEIN ALI
KHOSHBAVAR ROSTAMI**

Date :

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