

NON - SPECIFIC IMMUNE RESPONSES TOWARDS ASCORBIC
ACID SUPPLEMENTATION IN HYBRID CATFISH
(*Clarias gariepinus* x *C. macrocephalus*) FEED

DOAN NIAT PHUJONG

MASTER OF SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2007

**NON-SPECIFIC IMMUNE RESPONSES TOWARDS
ASCORBIC ACID SUPPLEMENTATION IN HYBRID CATFISH
(*Clarias gariepinus* x *C. macrocephalus*) FEED**

DOAN NHAT PHUONG

May 2007

Chairperson : Assoc. Prof. Abdul Ghafar and Prof. PhD

Member : Assoc. Prof. Abdul Muzaffar and Prof. PhD

Faculty : Faculty of Science and Technology

DOAN NHAT PHUONG

**Thesis Submitted in Fulfilment of the Requirement for the
Degree of Master of Science in the Faculty of Science and Technology
Universiti Malaysia Terengganu**

2007

1100054015

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Master of Science

**NON-SPECIFIC IMMUNE RESPONSES TOWARDS
ASCORBIC ACID SUPPLEMENTATION IN HYBRID CATFISH
(*Clarias gariepinus* x *C. macrocephalus*) FEED**

DOAN NHAT PHUONG

May 2007

Chairperson : Assoc. Prof. Mohd Effendy Abd. Wahid, PhD

Member : Assoc. Prof. Abol Munafi Ambok Bolong, PhD

Faculty : Faculty of Science and Technology

The effects of ascorbic acid (AA) on the growth, liver AA concentration, non-specific immune responses, and mortality of hybrid catfish were determined. Fishes were fed with diets of group 1 (0 mg AA/kg diet), group 2 (16.5 mg AA/kg diet), group 3 (33.3 mg AA/kg diet), group 4 (70.9 mg AA/kg diet), and group 5 (100.6 mg AA/kg diet) for 2, 4, 6, 8 and 10 weeks, respectively. By the end of experiment, mean body weight, wet weight gain, weight gain, and specific rate growth in the group 4 and 5 were significantly higher than those of the other groups ($p < 0.05$).

Prior to the challenge of *Aeromonas hydrophila* (*A. hydrophila*), there were significant increases ($p < 0.05$) in AA concentration in the liver of fish from the week 4 onwards. By the end of experiment, the group 3, 4 and 5 showed significantly higher ($p < 0.05$) liver AA concentrations than the group 2, but no significant difference was observed between them ($p > 0.05$). In contrast, there were significant decreases ($p < 0.05$) in liver

AA concentration when fishes were infected with *A. hydrophila*, but the group 3, 4 and 5 still maintained a high AA concentration in the liver.

In the present study, red blood cell (RBC), lymphocyte, thrombocyte, monocyte, and neutrophil were observed in the blood of hybrid catfish. Before challenged with *A. hydrophila*, there was significant decrease ($p < 0.05$) in diameter of RBC starting from the week 4, while monocyte, neutrophil showed a significant increase ($p < 0.05$) in their diameters from the week 8 onwards. Beside that, their diameters showed significant differences ($p < 0.05$) every sampling when fishes were injected with *A. hydrophila*. Diameters of lymphocyte and thrombocyte were not affected by the experimental diets and the bacterial infection ($p > 0.05$).

There was no significant effect ($p > 0.05$) of AA on the RBC number, but it was observed in the total white blood cell number from the week 8 onwards ($p < 0.05$). In addition, its effects on the numbers of lymphocyte and thrombocyte were not clearly defined in this study. Although its effects on the numbers of monocyte and neutrophil only showed in the week 8 of the non-infected experiment, it was observed in the week 8 and 10 of the experimental infection.

The O_2 - production, lysozyme concentration, ACH50 value, and IgM concentration were enhanced by increasing the AA level supplemented in diet, but significant differences were only shown from the week 8 onwards. However, after challenged with *A. hydrophila*, only IgM concentration was decreased by increasing the AA level supplemented in diet ($p < 0.05$). In general, the group 4 and 5 showed the best result on the immune parameters in this study.

The percent survival of *A. hydrophila* (PSA) was decreased by increasing the AA level supplemented in diet from the week 8 onwards. Both G4 and G5 had significantly lower PSA value than the other groups in the last 2 samplings. After challenged with *A. hydrophila*, the percent survival of fish was improved from 39.97% to 59.98% in the group 3, 4 and 5 for 8 and 10 weeks. In conclusion, diet containing 70.9 mg of AA/kg will improve the percent survival of fish.

July 2017

Supervisor : Prof. Mulya Mulya Kusumah, PhD, Widyaiswara

Asst. : Prof. Mulya Mulya Kusumah, Widyaiswara

Paralel : Fakultas Sains dan Teknologi Marinydia

Konsep asam lemak (AA) termasuk asam lemak, kemampuan AA pada hati, metabolisme, respon, dan kemampuan hati untuk lebih meningkatkan, serta diberi makan pada diet Kumpulan 1 (0mg AA/kg diet), Kumpulan 2 (19.5mg AA/kg diet), Kumpulan 3 (39.1mg AA/kg diet), Kumpulan 4 (70.9mg AA/kg diet) dan Kumpulan 5 (100.6mg AA/kg diet) masing-masing selama 1, 5, 8 dan 10 minggu. Pada akhir setiap periode panen, persentase hasil ikan, persentase hasil, kadar pertumbuhan ikan pada Kumpulan 4 dan 5 tidak lebih tinggi dan setara ($p > 0.05$) dibandingkan dengan Kumpulan 1-3.

Sebelum dan dengan menggunakan antibiotik di lingkungan, tindakan pencegahan yang benar ($p < 0.05$) persentase AA di dalam hati ikan berbeda dari minggu ke-4 dan seterusnya. Pada akhir setiap Kumpulan 3, 4 dan 5 telah menunjukkan kandungan AA hati yang signifikan tinggi ($p < 0.05$) dibandingkan dengan Kumpulan 2, tetapi tidak berbeda signifikan di antara minggu ($p > 0.05$). Sebaliknya, tindakan pencegahan yang utama pada Kumpulan 4 dan 5 tidak lebih ($p > 0.05$) apabila diaduk dengan *A. hydrophila* tetapi Kumpulan 3, 4 dan 5 masih menunjukkan kemampuan AA yang tinggi pada hati.

Abstrak tesis yang dikemukakan kepada senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

**TINDAKBALAS IMMUNITI NON-SPEKIFIK TERHADAP PENAMBAHAN
ASID ASKORBIK DALAM MAKANAN KELI HIDRID
(*Clarias gariepinus* x *C. macrocephalus*)**

DOAN NHAT PHUONG

May 2007

Pengerusi : Prof. Madya Mohd Effendy Abd. Wahid, PhD

Ahli : Prof. Madya Abol Munafi Ambok Bolong, PhD

Fakulti : Fakulti Sains dan Teknologi Malaysia

Kesan asid askorbik (AA) terhadap tumbesaran, kepekatan AA pada hati, tindakbalas non-spesifik, dan kematian keli hibrid telah ditentukan. Ikan diberi makan pada diet Kumpulan 1 (0mg AA/kg diet), Kumpulan 2 (16.5mg AA/kg diet), Kumpulan 3 (33.3mg AA/kg diet), Kumpulan 4 (70.9mg AA/kg diet) dan Kumpulan 5 (100.6mg AA/kg diet) masing-masing selama 2, 6, 8 dan 10 minggu. Pada akhir kajian, purata berat badan, pertambahan berat basah, pertambahan berat, kadar pertumbuhan khusus pada Kumpulan 4 dan 5 adalah lebih tinggi dan ketara ($p < 0.05$) berbanding dengan kumpulan-kumpulan lain.

Sebelum diuji dengan *Aeromonas hydrophila* (*A. hydrophila*), terdapat peningkatan yang ketara ($p < 0.05$) pada kepekatan AA di dalam hati ikan bermula dari minggu ke-4 dan seterusnya. Pada akhir kajian, Kumpulan 3, 4 dan 5 telah menunjukkan kepekatan AA hati yang signifikan tinggi ($p < 0.05$) berbanding dengan Kumpulan 2, tetapi tiada perbezaan signifikan di antara mereka ($p > 0.05$). Sebaliknya, terdapat pengurangan yang ketara pada kepekatan AA dalam hati ($p < 0.05$) apabila disuntik dengan *A. hydrophila*, tetapi Kumpulan 3, 4 dan 5 masih mengekalkan kepekatan AA yang tinggi pada hati.

Dalam kajian ini, sel darah merah, limfosit, trombosit, monosit dan neutrofil telah diperhatikan dalam darah ikan keli hibrid. Sebelum diuji dengan *A. hydrophila* terdapat pengurangan yang signifikan ($p < 0.05$) dalam diameter sel darah merah bermula dari minggu ke empat, sementara peningkatan yang signifikan ($p < 0.05$) pada diameter monosit dan neutrofil bermula dari minggu ke lapan dan seterusnya. Selain daripada itu, diameter mereka menunjukkan perbezaan yang signifikan ($p < 0.05$) setiap kali penyampelan dibuat pada ikan yang disuntik dengan *A. hydrophila*. Diameter limfosit dan trombosit pula tidak terjejas oleh diet yang diuji dan juga jangkitan bakteria ($p > 0.05$).

Tiada kesan AA yang signifikan pada bilangan sel darah merah ($p > 0.05$), tetapi kesan tersebut dapat dilihat pada bilangan keseluruhan sel darah putih daripada minggu ke lapan dan ke atas ($p < 0.05$). Sebagai tambahan, kesannya ke atas bilangan limfosit dan trombosit tidak dapat dipastikan. Walaupun kesannya ke atas bilangan monosit dan neutrofil hanya ditunjukkan dalam minggu ke lapan pada kajian ikan yang tidak dijangkiti, ianya telah dapat dikesan pada minggu ke lapan dan ke-sepuluh pada ikan yang dijangkiti.

Penghasilan O_2^- , kepekatan lisozim, nilai ACH50, dan kepekatan IgM telah meningkat dengan pertambahan tahap AA dalam diet, tetapi perbezaan ketara hanya dilihat dari minggu ke lapan dan seterusnya. Walau bagaimanapun, setelah diuji dengan *A. hydrophila*, hanya kepekatan IgM yang berkurangan ($p < 0.05$) walaupun tahap AA bertambah di dalam diet. Secara umumnya, Kumpulan 4 dan 5 telah menunjukkan keputusan terbaik ke atas parameter imun yang dikaji.

Kadar kemandirian *A. hydrophila* (PSA) berkurangan dengan peningkatan kandungan AA dalam diet makanan dari minggu ke lapan dan minggu seterusnya. Kedua-dua Kumpulan 4 dan 5 mempunyai PSA yang lebih rendah berbanding dengan kumpulan lain pada dua penyampelan terakhir. Setelah diuji dengan *A. hydrophila*, peratus ikan yang hidup meningkat dari 39.97% kepada 59.98% pada Kumpulan 3, 4 dan 5 untuk minggu ke lapan dan ke sepuluh. Kesimpulannya, kadar kemandirian ikan akan

meningkat dengan pemberian makanan yang mengandung diet sebanyak 70.9 mg AA/kg

First of all, I would like to express my grateful thanks to Assoc. Prof. Dr. Mohd Helmy Abd. Wahid, the Chairman of the Supervisory Committee for his available guidance, trust, and supervision during my Master program in the Universiti Malaysia Terengganu (UMT), Malaysia. Without his constant encouragement, this work would never be completed.

My grateful thanks are also extended to the Committee Member, Assoc. Prof. Dr. Ahmad Muhsin Ahmad Baheng for his helpful suggestions, constructive comments and meaningful contribution to this work. I would express my sincere thanks to Prof. Dr. Mohd Azam Abdul (Dean of the Graduate School), Prof. Dr. Aziz Hamzah bin Mohd Top (Dean of Faculty of Science and Technology), Dr. Siti Zaharah Abdulillah (National Fish Health Research Centre, Pongoh, Malaysia) and Dr. Jeney Galina (Research Institute for Fisheries, Aquaculture and Irrigation, Hungary) who helped me in every possible way.

I would also thank to Carole University for granting permission and allowing me to pursue my study. I acknowledge my gratitude to Assoc. Prof. Dr. Ngewon Ash Tann, Assoc. Prof. Dr. Ngewon Thach Phuong, Dr. Dana Thi Phuong Thanh, Dr. Thu Minh Tam, Mrs. Thi Thuan Dung, Mrs. Tran Thi Thuy Hoa, and my Colleagues for giving me their valuable advice and necessary assistance towards my research.

I wish to extend my most sincere gratitude and deep appreciation to all lecturers and staff members of the UMT, especially, to Mrs. Nur Hafizah Abdullah for the use of the