



UNIVERSITI PUTRA MALAYSIA

**EFFECT OF DIETARY ASCORBIC ACID ON WOUND HEALING,
SUSCEPTIBILITY TO AEROMONAS HYDROPHILA INFECTION AND
ITS SEQUENTIAL HEMATOLOGY IN THE AFRICAN CATFISH,
CLARIAS GARIEPINUS**

GREGORIA E. ERAZO

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By

GREGORIA E. ERAZO

**Thesis Submitted in Fulfilment of the
Requirement for the Degree of Master of Science
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LIST OF ABBREVIATIONS

| | | | | | | |
|--------|---|---|---|---|---|--------------------------------------|
| ASA | . | . | . | . | . | Ascorbic Acid |
| ANOVA | . | . | . | . | . | Analysis of Variance |
| CFU | . | . | . | . | . | Colony Forming Unit |
| H & E | . | . | . | . | . | Hematoxylin & Eosin |
| LSD | . | . | . | . | . | Least Significance Difference |
| mg/kg | . | . | . | . | . | Milligram per kilogram |
| MS 222 | . | . | . | . | . | Tricaine methane sulfonate |
| NADP | . | . | . | . | . | Nicotinamide Adenine Dinucleotide |
| NRC | . | . | . | . | . | National Research Council |



LIST OF FISH SPECIES

| Common Name | Scientific Name |
|----------------------------------|--------------------------------|
| African Catfish | <u>Clarias gariepinus</u> |
| Asian Catfish | <u>Clarias batrachus</u> |
| Atlantic Salmon | <u>Salmo salar</u> |
| Blue Tilapia | <u>Tilapia aurea</u> |
| Blue Catfish | <u>Ictalurus punctatus</u> |
| Common Carp | <u>Cyprinus carpio</u> |
| Coho Salmon | <u>Oncorhynchus kisutch</u> |
| Channel Catfish | <u>Ictalurus punctatus</u> |
| Fathead Minnow | <u>Pimephelales promelas</u> |
| Green Snakehead | <u>Channa punctatus</u> |
| Guppy | <u>Poecilia reticulata</u> |
| Indian Major Carp | <u>Cirrhina mrigala</u> |
| Japanese Flounder | <u>Paralichthys olivaceus</u> |
| Mexican Native Cichlid | <u>Cichlasoma urophthalmus</u> |
| Nile Tilapia | <u>Oreochromis niloticus</u> |
| Rainbow Trout | <u>Salmo gairdneri</u> |
| Red Sea Bream | <u>Chrysophrya major</u> |
| Snakehead | <u>Ophicephalus striatus</u> |
| Seabass | <u>Lates calcarifer</u> |
| Sockeye | <u>Oncorhynchus nerka</u> |
| Tilapia | <u>Oreochromis aureus</u> |



| | | | | | | |
|------------|---|---|---|---|---|-------------------------------|
| Turbot | . | . | . | . | . | <u>Scophthalmus maximus</u> |
| Walleyes | . | . | . | . | . | <u>Stizostedion vitreum</u> |
| Whitefish | . | . | . | . | . | <u>Coregonus lavaretus</u> |
| Yellowtail | . | . | . | . | . | <u>Seriola quinqueradiata</u> |



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By

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June 1997

Chairman : Prof. Dr. Mohd. Shariff Mohd. Din.

Faculty : Veterinary Medicine and Animal Science

Two experiments were conducted with African catfish, Clarias gariepinus (body weight, 80-150 g), under laboratory conditions. Fish were fed practical-type diets containing different levels of ascorbic acid (0, 60, 100, 300 and 700 mg/kg). In experiment 1, small surgical incisions were made in the dorsolateral musculature. Fish were sampled every 4hr over 14 days and histopathological assessment of the wound was carried out. The results showed that irrespective of vitamin levels, epidermis and dermis were almost completely healed except in fish fed deficient of ascorbic acid which exhibit delayed collagen deposition.



The ascorbic acid concentrations in liver increased significantly as dietary ascorbic acid concentrations increased. However, it gradually decreased until the 14th day of the experiment.

Fibroblast were present at 96h irrespective of the ascorbic acid level. Skin and muscle at the wound site were almost normal after 6 days in fish fed 60-700 mg of ascorbic acid/kg of diet.

In experiment 2, wounded fish were exposed to live Aeromonas hydrophila by immersion and sampled for histopathological evaluation for 28 days. Fish fed the ascorbic acid-free diet have slower wound repair compared with fish fed diet supplemented with ascorbic acid in the diet.

The performances of wounded fish fed diets deficient and supplemented with ascorbic acid were evaluated on the basis of their haematological parameters such as haematocrit (Hct), haemoglobin (Hb) content, total erythrocytes (TRBC), total leucocytes (TWBC) and differential leucocyte count which were done simultaneously in experiment 2. Haematocrit values and haemoglobin content varied with increasing levels of ascorbic acid level in the diets. No significant difference was found in differential leucocyte count. There were decreasing trends



of TRBC values with increasing ascorbic acid and an increase in TWBC in lower ascorbic acid levels.

None of the fish died during the experimental period and fish fed ascorbic acid-free diet did not exhibit any deficiency signs. The results of this study therefore indicate that the optimum level of ascorbic acid requirement lies between 60 to 700 mg ascorbic acid/kg diet.



Abstrak tesis yang dikemukakan kepada Senat Universiti
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keperluan untuk mendapat Ijazah Master Sains.

**KESAN ASID ASKORBİK DIETARI DALAM PENYEMBUHAN LUKA,
KEBOLEHTAHANAN TERHADAP JANGKITAN AEROMONAS HYDROPHILA,
DAN KESAN HEMATOLOGINYA KEPADA KELI AFRIKA,
CLARIAS GARIEPINUS.**

oleh

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Jun 1997

Pengerusi : Prof. Dr. Mohd. Shariff Mohd. Din
Fakulti : Kedoktoran Veterinar dan Sains Penternakan

Dua eksperimen menggunakan keli Afrika, Clarias gariepinus (berat badan, 80-150g), telah dijalankan di makmal. Ikan diberi makan diet praktikal yang mengandungi tahap asid askorbik yang berbeza (0, 60, 100, 300 dan 700 mg/kg). Dalam eksperimen 1, satu luka kecil telah dilakukan di bahagian otot dorsolateral. Ikan disampel setiap 4 jam selama 14 hari dan penilaian histopatologi luka telah dijalankan. Keputusan menunjukkan bahawa kesamaluka pada epidermis dan dermis hampir sembuh pada semua rawatan kecuali pada ikan yang diberi makanan tanpa asid askorbik yang mana menunjukkan sintesis kolagen yang perlahan.



Kepekatan asid askorbik dalam hati meningkat apabila kepekatan dietari asid askorbik meningkat. Walau bagaimanapun, kepekatan pada semua rawatan menurun secara perlahan sehingga hari ke-14 ujikaji.

Fibroblast dapat dilihat pada 96j pada semua paras asid askorbik. Kulit dan otot pada tempat perlukaan hampir kembali normal selepas 6 hari di kalangan ikan yang diberi makan 60-700 mg asid askorbik/kg makanan.

Dalam eksperimen 2, ikan yang dilukakan didedahkan kepada Aeromonas hydrophila hidup melalui rendaman dan disampel untuk penilaian histopatologi selama 28 hari. Ikan yang diberi makanan tanpa asid askorbik mengalami penyembuhan luka yang lambat berbanding dengan ikan yang diberi makanan yang ditambah dengan asid askorbik.

Prestasi ikan yang dilukakan yang diberi makanan dengan atau tanpa asid askorbik tambahan dinilai berdasarkan kepada parameter hematologi seperti nilai hematokrit (Hct), hemoglobin (Hb), jumlah sel darah merah (TRBC), jumlah sel darah putih (TWBC) dan kiraan leukosit yang dilakukan serentak dalam eksperimen 2. Nilai hematokrit dan kandungan hemoglobin berubah dengan peningkatan paras asid askorbik dalam makanan. Tiada perubahan ketara yang didapati dalam kiraan leukosit. Kadar TRBC didapati menurun dengan peningkatan asid askorbik manakala TWBC meningkat pada kadar asid askorbik

yang rendah.

Tiada ikan mati semasa eksperimen manakala ikan yang diberi makanan tanpa asid askorbik tidak pula menunjukkan sebarang tanda kekurangan. Keputusan kajian ini menunjukkan bahawa paras optimum bagi keperluan asid askorbik untuk penyembuhan luka adalah di antara 60 hingga 700 mg asid askorbik/kg makanan.



CHAPTER I

GENERAL INTRODUCTION

Vitamins supplements are not all essential to fishes (Boonyaratpalin et al., 1989). Only those that cannot be synthesized by the fish must be supplemented in diets. This is especially true for ascorbic acid, a water-soluble vitamin.

The need to incorporate ascorbic acid (vitamin C) in fish diets has been demonstrated in rainbow trout (Halver et al., 1969; Navarre and Halver, 1989; Cho and Cowey, 1991; Dabrowski and Blom, 1994), coho salmon (Halver et al., 1969), channel catfish (Lovell, 1973; Andrews and Murai, 1975; Li and Lovell, 1985; Liu et al., 1989; El-Naggar and Lovell, 1991), red sea bream (Yano et al., 1988), snake head (Mahajan and Agrawal, 1979), carp (Mahajan and Agrawal, 1980), Asian catfish (Butthep et al., 1985), Nile tilapia (Soliman et al., 1993), and sea bass (Boonyaratpalin et al., 1989).

Ascorbic acid, which acts primarily as a reducing agent, is involved in many biological functions (Masumoto et al., 1991). It hastens wound healing in tilapia

