UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL ATTRIBUTE AND DISEASE RESISTANCE OF CATFISH Clarias gariepinus (Burchell 1822) SUPPLEMENTED WITH DIFFERENT RATIONS OF SPIRULINA Arthrospira platensis

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By

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Spirulina *Arthrospira platensis* is practically used as a supplement in nutrition due to its high nutritional content. Improving the feed formulation by combining the commercial fish feed with spirulina to the cultured fish might elevate the fish growth performance, nutritional value and increase its resistance towards bacterial infection. This study was conducted to determine the growth performance, nutritional value and disease resistance of catfish *Clarias gariepinus* (Burchell 1822) supplemented with different ration of spirulina, *A. platensis.* Five experimental diets were prepared by varying the percentage of spirulina in the fish feed containing 0% (control), 1%, 3%, 5% and 7% respectively of locally grown spirulina. A total of 30 fry (2.62±0.04gof fish initial total body weight; 7.09±0.05 cm of total body length) were stocked per cage and in triplicate. Each diet was fed to the fry twice daily at 5% of fish body weight for 90 consecutive days. Growth performances were determined every fortnight. The proximate analysis of spirulina, experimental diets and catfish fillet was done according to the method of AOAC, 1990. The catfish were then intraperitoneally injected with 10^7 CFU/ml of virulent *Aeromonas hydrophila* on day 91, and monitored until day 104. Catfish with diet of 7% spirulina produced significantly higher ($p<0.05$) in weight gain and specific growth rate compared to control, but not with other treatment groups. Standard length, percent of survival, feeding efficiency of the catfish increased with the increasing percentage of spirulina inclusion but was not significantly different ($p>0.05$) between all the diet groups. The feed conversion rate marginally decreased as spirulina inclusion increased in the diet, but not significantly different ($p>0.05$) between all the treatment groups. The gonado somatic index value was the highest in catfish with diet 7% spirulina but not significantly different ($p>0.05$) between all the treatment groups. The hepatosomatic index and viscerosomatic index value were also not significantly different ($p>0.05$) between all the treatment groups. The proximate compositions of the locally grown dried spirulina showed the highest content was the crude protein with 63.07±0.02% ($p<0.05$) followed by the carbohydrate content, ash, moisture, crude lipid and lastly crude fiber. Generally, as the percent of spirulina in the diet increase the proximate composition of the experimental

diets were significantly increased ($p<0.05$) for the crude protein and significantly lower ($p<0.05$) for the crude fiber content. The moisture, ash, crude lipid and carbohydrate showed no significant ($p>0.05$) different between all the experimental diet groups. The content of proximate composition of catfish fillet showed at par to the control group with no significant different ($p>0.05$) between all diet groups except the crude fiber content that were significantly increased ($p<0.05$) with increasing percent of spirulina until at maximum of 3% of spirulina inclusion and there was no significant different ($p>0.05$) at 5% and 7% of spirulina diet. The percentage of catfish survive following *A. hydrophila* infection were significantly low ($p<0.05$) in control with 36.67% but not with other treatment groups range from 70.00% to 73.33%. The white blood cell count value was significantly higher ($p<0.05$) in group supplemented with 7% spirulina whereas the red blood cell, hemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration value showed no significant different between the treatment groups. This study conclude that the catfish supplemented with spirulina as low as 1% resulted in optimum disease resistance against the bacterial infection of *A. hydrophila* which helps in preventing mass mortality of catfish although the spirulina does not accelerate the growth performance and nutritional value of the *C. gariepinus*.

Keyword: *Clarias gariepinus*, *Aeromonas hydrophila*, *Arthrospira platensis*, growth performance, disease resistance

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

ATRIBUT BIOLOGI DAN RINTANGAN PENYAKIT IKAN KELI *Clarias gariepinus* DITAMBAH DENGAN NISBAH BERBEZA SPIRULINA *Arthrospira platensis*

Oleh

NOR FATIHAH MOHD NASIR

Oktober 2017

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Spirulina*Arthrospira platensis*lazimnya digunakan sebagai tambahan dalam pemakanan kerana kandungan khasiatnya yang tinggi.Meningkatkan rumusan makanan yang menggabungkan makanan ikan komersial dengan spirulina kepada ikan yang diternak mungkin dapat meningkatkan prestasi pertumbuhan ikan, nilai khasiat pemakanan dan meningkatkan daya tahan ikan daripada jangkitan bakteria.Kajian ini dijalankan untuk menentukan prestasi pertumbuhan, nilai nutrisi ikan dan rintangan penyakit ikan keli *Clarias gariepinus* (Burchell 1822) yang ditambah dengan catuan berbeza spirulina, *A. platensis*. Lima diet eksperimentasi yang disediakan dengan memanipulasi peratusan spirulina dalam makanan ikan masing-masing mengandungi 0% (pemalar), 1%, 3%, 5% dan 7% spirulina keluaran tempatan. Sebanyak 30 ekor anak ikan keli Afrika (2.62±0.04g jumlah berat ikan awal; 7.09±0.05 sm jumlah panjang ikan) telah ditempatkan dalam setiap sangkar secara triplikasi. Diet yang disediakan diberi makan kepada anak ikan dua kali sehari 5% dari berat badan ikan selama 90 hari.Kadar pertumbuhan ditentukan setiap dua minggu.Analisis proximat spirulina, diet eksperimen dan fillet ikan keli dilakukan mengikut kaedah AOAC 1990.Ikan keli kemudiannya disuntik secara intraperitoneal dengan 10^7 CFU/ml *A. hydrophila* pada hari ke-91 dan pemerhatian dilakukan sehingga hari ke-104. Keli dengan diet 7% spirulina menghasilkan perbezaan yang signifikasi ($p<0.05$) dalam jumlah pertambahan berat badan dan kadar pertumbuhan tertentu berbanding pemalar, tetapi tidak dengan kumpulan diet yang lain. Panjang standard, peratus hidup, FE ikan keli meningkat dengan pertambahan peratusan spirulina di dalam diet tetapi tidak berbeza secara signifikan ($p>0.05$) antara semua kumpulan diet. Nilai kadar penukaran makanan menunjukan sedikit penurun apabila peratus spirulina meningkat di dalam diet, tetapi tidak berbeza secara signifikan ($p> 0.05$) antara semua kumpulan diet. Nilai gonad somatik indeks adalah yang tertinggi dalam ikan keli dengan diet 7% spirulina tetapi tidak ketara berbeza ($p>0.05$) antara semua kumpulan rawatan. Nilai hepatosomatik indeks dan viscerosomatik indeks juga tidak berbeza secara ketara ($p>0.05$) antara semua kumpulan rawatan. Komposisi proksimat spirulina kering tempatan menunjukan kandungan tertinggi ialah protein mentah dengan 63.07±0.02%

($p<0.05$) diikuti dengan kandungan karbohidrat, abu, kelembapan, lipid mentah dan akhir sekali ialah serat mentah. Secara umumnya, apabila peratus spirulina meningkat dalam diet komposisi proksimat meningkat bagi kandungan protein mentah ($p<0.05$) dan menurun bagi kandungan serat mentah ($p<0.05$). Nilai kandungan kelembapan, abu, lipid mentah dan karbohidrat tidak menunjukkan perbezaan yang signifikan ($p>0.05$) antara semua kumpulan diet eksperimen. Kandungan komposisi proksimat fillet ikan keli kesemua kumpulan diet menunjukan nilai yang setanding dengan kumpulan kontrol tanpa sebarang perbezaan ($p>0.05$) kecuali kandungan serat mentah yang meningkat secara ketara ($p<0.05$) dengan peningkatan peratus spirulina sehingga 3% campuran spirulinadan tiada perbezaan yang signifikan ($p>0.05$) pada kadar 5% dan 7% campuran spirulina. Peratusan hidup ikan keli susulan jangkitan *A. hydrophila* adalah sangat rendah ($p<0.05$) dalam kumpulan kawalan dengan 36.67% tetapi tidak dengan kumpulan rawatan lain dimana mempunyai peratus hidup dari 70.00% sehingga 73.33%. Nilai kiraan sel darah putih adalah lebih tinggi ($p<0.05$) dalam kumpulan ikan keli yang diberi makan dengan 7% spirulina manakala nilai sel darah merah, haemoglobin, isipadu sel padat, min isi padu korpuskel dan min kepekatan korpuskel haemoglobin menunjukkan tiada perbezaan yang signifikan ($p>0.05$) di antara kumpulan rawatan. Kajian ini menyimpulkan bahawa ikan keli yang diberi makanan tambahan spirulina pada 1% dapat mencapai daya tahan penyakit yang optimum dalam melawan penyakit jangkitan bakteria *A. hydrophila* dimana ia dapat membantu dalammengurangkan jumlah kematian ikan keli secara besar-besaran walaupun spirulina tidak meningkatkan prestasi pertumbuhan dan nilai khasiat *C. gariepinus*.

Kata kunci: *Clarias gariepinus, Aeromonas hydrophila, Arthrospira platensis*, prestasi pertumbuhan, daya tahan penyakit

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I certify that a Thesis Examination Committee has met on 12 October 2017 to conduct the final examination of Nor Fatihah binti Mohd Nasir on her thesis entitled "Biological Attributes and Disease Resistance of Catfish Clarias gariepinus (Burchell 1822) Supplemented with Different Rations of Spirulina Arthrospira platensis"in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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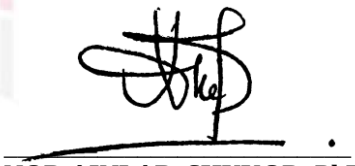

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

A. hydrophila	*Aeromonas hydrophila*
A. platensis	*Arthrospira platensis*
BAU	Bangladesh Agricultural University
bc	Blood congestion
bv	Blood vessel
ca	Central artery
cv	Central vein
d	Degeneration
DPX	Distyrene, plasticizer, xylene
DOF	Department of Fisheries
et al.	Latin phrase means 'and others'
FCR	Feed conversion rate
FE	Feeding efficiency
FOI	French Oil Institute
g	Glomerulus
GSI	Gonadosomatic index
h	Hepatocytes
Hb	Hemoglobin
hyt	Hypertrophy
H&E	Hematoxylin & eosin
IPGSR	Institute of Post-graduate Studies and Research Laboratory
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
MMC	Melanomacrophage centre
n	Necrosis
PCV	Packed cell volume
RBC	Red blood cell
rc	Renal corpuscle
rp	Red pulp
SAC	Siam Algae Co. Ltd
SGR	Specific growth rate
SL	Standard length
TBI	Total body increase
tb	Trabeculae
VSI	Viscerosomatic index
wp	White pulp
WBC	White blood cell

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Aquaculture industry in Malaysia has become an important activity as it increases the local production of food since the Seventh Malaysia Plan (1996-2000) and benefits the economy of the country (FAO, 2014). Aquaculture industry also has been identified as potential export earner after oil palm and rubber, and also as one of the most profitable in terms of income per hectare per annum and return to investment (FAO, 2014).

Presently, there are several species of fish cultured intensively including catfish. Catfish *Clarias* spp. recorded the highest production of cultured freshwater fish in Malaysia. The total production of this fish was approximately 50,534 tonnes, with estimated retail value of RM 314 millions (DOF, 2013). Most of this fish were cultured in freshwater ponds (48,422 tonnes; 95.8%), followed by cemented tanks (1,242 tonnes; 2.5%), canvas tanks (354 tonnes; 0.7%) and others. It is predicted that the production of catfish will increased in the future, due to the demand, high prices and farming technology (DOF, 2013).

Intensive culture of fish that fed with land based feed source has become problem to the aquaculture farming industry (Avnimalech, 2009). According to Hemarswarya et al. (2011), almost 40-60% of aquaculture production depends on the commercial feed. The land based feed source such as corn meal, soy meal and other feed additives which is non-living diets not only expensive, but the growth of the fish are slow, prone to disease and cause high mortalities compared to those fed with live diet (Ponis et al., 2003). Hence, the replacement or the substitution of the land based feed source required an alternative feeds that much cheaper and promote the growth of the farmed fish with its high nutritional value and at the same time reduce the rate of mortality.

There were studies proved that the use of aquatic origin natural food which is microalgae such as spirulina *Arthrospira platensis* as fish feed can provide nutrient to the aquatic farmed animals as it were known to have good nutritional value (Brown, 2002; Natrah et al., 2007), probiotic effects and stimulate the immunity of the cultures aquatic animals (Muller-Feuga, 2000). Numerous studies have shown that feed supplementation and improved feed formulation can alleviate the performance of the fish's growth and disease resistance, thus these provide alternative to the problems regarding to aquaculture production.

Study by Otto & Gross (2004) showed that spirulina or its extracts accelerate the development of the immune system of many animals especially during early stages of their lives. Certik & Shimizu (1999) stated the large profile of natural vitamins, minerals and essential fatty acids content of the spirulina positively improved the

immune response of the animal fed with spirulina. Other than that, the bioactive components in spirulina give the positive health effects. The immune enhancement effect through its anti-viral and anti-bacteria properties is one of the promising applications of using spirulina as feed to the reared or cultured animals.

Disease outbreaks caused by *Aeromonas hydrophila*in intensive culture of catfish has become a problem to the aquaculture industry as it affects the freshwater fish production (Salah, 2013). For example, there was a report in March 2008 on mass mortality of cultured African catfish *Clarias gariepinus* in a floating cage culture system located at Manir River, Terengganu, Malaysia, due to *A. hydrophila* infection, which caused high economic losses to the industry (Najiah et al., 2009). *Aeromonas hydrophila* were known as a species of bacterium that can be found in all freshwater environments and brackish water (FDA, 2015). The infection of *A. hydrophila* on catfish may be transmitted through the water, diseased and healthy fish and other affected vertebrates. The infection can cause sudden death of the infected host, hemorrhagic septicemia including bloated appearance, skin ulcerations and pale gills (Laith & Najiah, 2013).

Thus, this study will evaluate the utilization of local spirulina as fish feed supplement and maximized the application of its benefit as food source for cultured aquatic animal. Moreover, this study also will try to reduce the reliance on the land based feed source and the expensive fish meal. It is expected that the catfish that fed with local spirulina will improved their growth performances, nutritional value and disease resistance towards bacterial diseases.

1.2 Problem statement

Intensive aquaculture practices that have been applied to fulfill the demand of consumer on the aquaculture production have raised a lot of problems. The first is about the feed given to the cultured fish. The farmers tend to feed the catfish or hardy fishes with cheaper and low quality of feed such as the chicken offal, trash fish and carcass due to expensive commercial fish feed. There was a case in Perak, Malaysia, when freshwater fish were fed with waste from pig industries and carcass of pigs or piglets (Bernama, 2017). This situation rise an issue concerning with halal food source to Muslim in Malaysia and make the catfish less feasible to the consumer.

The second problem is the unhealthy and slow growth of the catfish. This constraint related to the low quality of "farm-made feed" given to the cultured fish. The term "farm-made feed" refer to the processed carcasses into the fish feed. Inadequate nutrient for the growth and rancid condition of the offal and carcass can cause the fish prone to disease besides the growth of the fish slow and stunned (Chinabut, 2002).

The third problem is the disease outbreak. Disease outbreak in aquaculture often related with high stoking density or the intensive farming practices due to the efficiency in transmitting the infectious diseases which become a primary limiting factor of fish

production (Leung & Bates, 2013). As for the freshwater fishes, *Aeromonas hydrophila, Flexibacter, Edwardsiella* sp. and *Pseudomonas* are the most common cause of bacterial disease (Siti Zahrah, 1992). In fact, the distributions of A. hydrophila are reported to be present in Malaysia also in neighboring country such as Thailand and Indonesia (OIE, 2009). In the case of catfish culture, catfish is the host to the bacterial fish pathogen *A. hydrophila* (Laith & Najiah, 2013). It is a concern that disease outbreak caused by this bacterium will cause huge profit losses as catfish were been cultured intensively and recorded as highest produced freshwater fish in Malaysia (DOF, 2015). Besides being a most common cause of bacterial disease, *A. hydrophila* is an opportunistic bacterium that can be found in various water bodies worldwide which often related with severe disease to the infected fish (Janda & Abbott, 2010). The infection of *A. hydrophila* usually will cause mass mortality of the reared fish and showed bloated appearance, visible hemorrhagic lesion, ulceration on the fish's body surface or skin also the rotting of fins and tail (Miyazaki & Jo, 1985; Wolf, 1988). Besides, the multiplication of bacteria in the intestine of the fish will cause a haemorrhagic mucous-desquamative catarrh (Aoki, 2016). The *A. hydrophila*'s toxic metabolites induce toxaemia as it had been absorbed by the intestine and the stomach's submucosa experience the capillary haemorrhage (Aoki, 2016). Study that have been conducted proved that the disease outbreak by the bacterial disease often related with poor environment condition such as low dissolved oxygen, sudden change in temperature, stressors include the overcrowding and rough handling (Noga, 2010; Steven et al., 2017). Although catfish is a hardy species that can withstand stress and low water quality, as they develop a disease usually it has been too late for the remedial action as the outbreak already serious (Sayuthi, 1993). To date, disease control measure involving the usage of antibiotics or chemotherapeutics for treatment also probiotics and vaccines for preventive measures which requires better choice of disease control strategy as the demand of world market require healthy aquaculture products from farm to table (Chinabut & Puttinaowarat, 2005). The application of using chemical, drugs and antibiotics in the disease treatment rise several major issues include the impact to the environment as the release of antibiotics in large quantities, high residue levels of approved or unapproved antibiotics presence which lead to the development of bacteria that become more resistance to the developed antimicrobial drug which possess threat not only to the aquaculture but also human health. Vaccination in preventing the disease outbreak is proven to be highly effective and often applied to high economic value of fish species such as salmon. However, in developing vaccine for specific disease require time and money investment also the fish species farmed economic value need to be taken account for (Sommerset et al., 2005). The usage of probiotic in preventive measure has been used extensively in aquaculture to increase the disease resistance of the fish against the bacterial disease, appetite improvement and enhance the growth of the fish (Newaj-Fyzul et al., 2014). Hence the environmentally-friendly, highly effective and low cost alternative is required to reduce the problems in control and management of disease outbreak in the aquaculture.

1.3 Objectives

The main objectives of this study are:

1. To determine the growth performances of catfish *Clarias gariepinus* supplemented with different ration of spirulina *Arthrospira platensis*
2. To determine the proximate composition of catfish *Clarias gariepinus* supplemented with different ration of spirulina *Arthrospira platensis*
3. To determine the disease resistance of catfish *Clarias gariepinus* supplemented with different ration of spirulina against *Aeromonas hydrophila* infection

1.4 Hypothesis

The hypotheses of this study are:

1. Catfish *Clarias gariepinus* that are supplemented with spirulina *Arthrospira platensis* will have better growth performance compared to catfish that are not supplemented with spirulina
2. The proximate composition of catfish *Clarias gariepinus* supplemented with spirulina *Arthrospira platensis* will be increased compared to catfish that are not supplemented with spirulina
3. Addition of spirulina *Arthrospira platensis* in the catfish *Clarias gariepinus* diet may enhanced the disease resistance against *Aeromonas hydrophila* infection

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LIST OF PUBLICATION

Nor Fatihah Mohd Nasir, Mohammad Noor Amal Azmai, Hishamuddin Omar, Ahmad Ismail. 2017. Supplementation of spirulina (*Arthrospira platensis*) improves physical activeness level of catfish (*Clarias gariepinus*). International Journal of Biosciences. 11(5): 146-150.

Nor Fatihah Mohd Nasir, Mohammad Noor Amal Azmai, Hishamuddin Omar, Ahmad Ismail. 2017. Spirulina, *Arthrospira platensis* supplementation in catfish, *Clarias gariepinus*, reduces susceptibility to Aeromonas hydrophila infection. Paper presented at International Conference on Advance in Fish Health 2017 (ICFISH 2017), 4-6 April 2017 Office of the Deputy Vice Chancellor (Research and Innovation), Universiti Putra Malaysia, UPM Serdang, Selangor Malaysia.