UNIVERSITI PUTRA MALAYSIA

LARVAL NUTRITION OF SILVER BARB BARBODES GONIONOTUS WITH AN EMPHASIS ON PROTEIN-ENERGY REQUIREMENTS AND FEEDING STRATEGY

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By

CARINA MIRANDA TAYAG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

To my beloved parents, Carlito Tayag and Delinia Tayag, for the love, encouragement, prayers and for molding me through the years to become the person I am right now.

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chairman: Associate Professor Dr. Mohd. Salleh Kamarudin, Ph.D.

Faculty: Agriculture

A series of experiments were conducted to determine the possibility of rearing silver barb larvae on an artificial diet. Prior to the evaluation of artificial diet in rearing silver barb larvae, a study was conducted to determine the changes in the amino acid profile of silver barb from freshly stripped eggs to newly hatched larvae. The result showed that the amino acid pattern changed according to the developmental stages of the fish larvae.

The optimum protein requirement of silver barb larvae was determined using five isocaloric diets with varying protein levels (40, 45, 50, 55 and 60% protein). The results showed that the larval growth was highest when they were fed a diet containing 55% protein followed by those fed with 50, 45, 60 and 40% protein. Although the highest survival was obtained among larvae fed on a 50% protein diet, it was not significantly different ($P>0.05$) from the survival of those fed on a 55% protein diet.

In the following study, the optimal protein-energy requirement of silver barb larvae was determined. Six experimental diets containing two levels of protein (50% and 55% protein) and three energy levels (18.8, 19.7 and 20.5 kJ g^{-1}) were evaluated. In this study, larvae fed a diet containing 50% protein with 19.7 kJ g^{-1} energy obtained the best growth and highest survival. This level was found to be optimum for growth and maintenance of the larvae.

An experiment was also conducted to find the best feeding rate for silver barb larvae. The present study showed a significant relationship between feeding rate and growth of the larvae. Based on growth, feed efficiency and survival, an optimal feeding rate of 45% BW d^{-1} was found for silver barb larvae.

Lastly, this study revealed that silver barb larvae are visual feeder. Feeding the larvae throughout the day (day and night) did not give any beneficial effect for the larvae and resulted in lower growth and survival. This study suggested that feeding the larvae four times during daytime was the best feeding frequency for silver barb larviculture.

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

PEMAKANAN LARVA LAMPAM JAWA *BARBODES GONIONOTUS* DENGAN PENEKANAN TERHADAP KEPERLUAN PROTEIN-TENAGA DAN STRATEGI PEMBERIAN MAKANAN

Oleh

CARINA MIRANDA TAYAG

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Beberapa siri eksperimen telah dilakukan untuk mengkaji kemungkinan menternak ikan lampam jawa menggunakan makanan buatan. Sebelum penilaian makanan buatan dalam pengkulturan larva lampam Jawa dilakukan, satu kajian telah dijalankan untuk mengetahui perubahan yang berlaku terhadap profil asid amino yang terkandung dalam telur yang baru dikeluarkan induk sehingga larva menetas. Kajian menunjukkan asid amino berubah mengikut tahap perkembangan larva ikan tersebut.

Keperluan protein optimum larva lampam Jawa ditentukan menggunakan lima diet isokalori dengan aras protein yang berbeza (40, 45, 50, 55 dan 60% protein). Keputusan menunjukkan bahawa pertumbuhan larva tertinggi didapati apabila larva diberi diet yang diberi mengandungi 55% protein dan diikuti larval yang diberi yang mengandungi 50, 45, 60 dan 40% protein. Walaubagaimanapun, larva yang diberi diet 50% protein menunjukkan kemandirian yang tertinggi tetapi tidak ketara berbeza ($P>0.05$) dari larva yang diberi diet mengandungi 55% protein.

Keperluan protein-tenaga optimum bagi larva lampam Jawa ditentukan dalam kajian berikutnya. Enam diet ujian yang mengandungi dua aras protein (50% dan 55% protein) serta tiga aras tenaga (18.82, 19.66 dan 20.5 kJ g^{-1}) telah diuji. Dalam kajian ini, larva yang diberi diet yang mengandungi 50% protein dengan 19.66 kJ g^{-1} menunjukkan pertumbuhan terbaik serta kemandirian yang tertinggi. Aras ini didapati optimum untuk pertumbuhan dan penyenggaraan larva.

Satu eksperimen juga telah dilakukan untuk menentukan kadar pemberian makanan yang terbaik untuk larva lampam Jawa. Kajian menunjukkan hubungan ketara antara kadar pemberian makanan dengan pertumbuhan larva. Berdasarkan pertumbuhan, kecekapan makanan serta kemandirian, kadar pemberian makanan optimum bagi larva lampam Jawa adalah 45% berat badan $hari^{-1}$.

Kajian ini menunjukkan bahawa larva lampam Jawa mencari makanan menggunakan penglihatan. Pemberian makanan kepada larva sepanjang hari (pada waktu siang dan malam) tidak memberikan sebarang kesan lebih baik terhadap larva malahan menyebabkan pertumbuhan dan kemandirian yang rendah. Kajian ini mencadangkan pemberian makanan sebanyak empat kali sehari pada waktu siang adalah lelerapan pemberian yang terbaik untuk pengkulturan larva lampam Jawa.

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ANOVA	Analysis of Variance
AOAC	Association of Analytical Chemist
BSA	Bovine Serum Albumin
BW	Body weight
CPE	Carp pituitary extract
CRD	Complete randomized design
DMRT	Duncan's multiple range test
DO	Dissolve oxygen
Expt.	Experiment
FCR	Feed conversion ratio
g	Gram
kJ	Kilo joule
L	Liter
N	Normality
P:E	Protein-to-energy ratio
PER	Protein efficiency ratio
SAS	Statistical Analysis System
SGR	Specific growth rate
UPM	Universiti Putra Malaysia
YSI	Yellow Spring Instrument
µm	Micrometer
EAA	Essential Amino Acid
FAA	Free Amino Acid

CHAPTER I

INTRODUCTION

Background of the Study

In the last ten years, aquaculture has been the fastest growing primary industry worldwide, with 39.4 million tons produced in 1998 (Tacon and Forster, 2000). The fishery sector in Malaysia plays an important role in the national economy in terms of income, foreign exchange and employment (Abu Talib and Alias, 1997). It also supplies 60% of the total animal protein intake of the population. The demand of fish, as the main source of protein is expected to increase from an annual consumption of 630,000 tons to over 1,580,000 tons by the year 2010 (DOF, 1995). As the fishery products from the sea are not expected to increase any further, aquaculture will assume a greater role to increase fish supply.

Barbodes gonionotus is one of the important species of aquaculture in Bangladesh (Hussain et al., 2002), Thailand (Wee and Ngamsnae, 1987; Koedprang and Na-Nakorn, 2000), Indonesia (Pongthana et al., 1999) as well as in Malaysia (Soong, 1963). It was originally introduced from Indonesia for pond culture in Malaysia during 1950's (Soong, 1963). It contributes 17.5% of the total output from freshwater ponds with a production of 2,505 tons in 1992 (Kechik, 1995). However, a reduction on the volume produced was observed in year 2000 and it accounts for approximately 4% only of the total freshwater fish produced in Malaysia (FAO, 2002). This reduction might be due to the lack of information in culturing this fish as well as in larval rearing which was believed to be the major bottleneck in fish production. This fish was categorized under Cyprinidae, the biggest Family of freshwater fish and its local name is lampam

Jawa (Mohsin and Ambak, 1983). This fish can be cultured in seasonal (roadside ditches, backyard ponds and rice fields) and perennial (tanks and ponds) water bodies often in combination with other species of carps (Hussain et al., 2002). It grows to a marketable size within 8-12 months but matures at 4-6 months of age (Koedprang and Na-Nakorn, 2000).

Silver barb was found to be a potential pituitary donor in inducing other species of fish to spawn in captivity (Kottelat, 1998). With regard to this, the artificial propagation of this fish was extensively practiced and was established since 1967 (Tangtropiros et al., 1990). However, even though the induced spawning of this fish had been carried out for more than 3 decades, studies on the larval rearing of this fish was in idle until recently. Jahangard (2003) reared this larvae using live food and he further noted that this larvae can be reared on alternative live food such as nematodes. However, by merely feeding the larvae with live food is not enough to determine their nutritional needs. This enigma gave birth to the field of larval nutrition. In conjunction to this, a noble research had been generated to develop an artificial diet as an economic live food alternative. Moreover, Rosenlund et al. (1997) stated that microdiet offers the opportunity to introduced nutrients that are not present in the live food.

Long before this new field evolved, most researchers have focused in determining live foods suitable for fish larvae. Later on, they studied the possibility of rearing fish larvae on formulated diet to minimize or eliminate the use of live food. Since then, a lot of studies have been done and results are often contradicting from each other. Nutritional requirements and larval rearing either marine or freshwater fish had been carried out through the years. Their findings suggested that fish larvae cannot be reared on an artificial diet alone and that live food must be supplied at their early life

stages (Dabrowski and Glogowski, 1977; Kestemont and Stalmans, 1992; Lazo et al., 2000). However, these findings were in contrast to the results found by several researchers wherein they suggested that fish larvae could be reared on artificial diet alone without compromising their growth and survival (Dabrowski et al., 1985; Zambonino Infante and Cahu, 1994; Diaz et al., 1997; Cahu et al., 1998; Gisbert and Williot, 2002).

Silver barb is a cyprinid fish where common carp (*Cyprinus carpio*) belongs. Common carp larvae showed good performance when only fed artificial diet, indicating that live food can be completely eliminated on the larval rearing of this fish (Alami-Durente et al., 1991; Charlon and Bergot, 1994; Kestemont, 1995). With this finding, the feasibility of rearing silver barb larvae on an artificial diet alone and determining the feeding strategy has yet to be defined. This study would also be of help to mitigate the problems often encounter in hatchery such as high mortality and high production cost which mainly comes from the production of live food and later could expand the aquaculture industry of silver barb.

Statement of the Problem

This millennium is faced with a challenge of producing more fish for the increasing population of the world, particularly in Southeast Asia. Aquaculture is widely recognized as viable and profitable enterprise worldwide. It will continue to grow and supply an increasingly larger percentage of fishery products consumed because the oceans, seas and rivers are inadequately managed by human and their yield is unpredictable (Lovell, 1988). With the increasing numbers of farmers shifting from agriculture into an aquaculture venture and with the modification of culture system, a

consistent supply of quality seedstock from the hatchery must be obtained to meet the demand of the farmers. However, the availability of quality seedstock has been one of the most critical factors in the commercial production of fin fishes.

Presently, the initial feeding of fish larvae in intensive condition depends on the supply of live prey such as rotifers and *Artemia*. In an attempt to increase the nutritional value of rotifers and *Artemia*, they are typically fed polyunsaturated fatty acid (PUFA) rich diets prior to being fed to larval fish. However, this practice further increased the production cost. The nauplii of *Artemia salina* are obtained from durable eggs collected in the natural environment and were sold at high price. Although *Artemia* certainly holds many benefits for larviculture, there are several disadvantages. First, *Artemia* is expensive. Second, if used as a sole food, it does not satisfy the nutritional requirements of larvae and consequently needs to be enriched at a considerable expense (Bernabe and Guissi, 1982). *Artemia* have resulted in high degree of abnormalities such as malpigmentation and incomplete eye migration in flatfish (Tonheim et al., 2000). The mortality rate of fish larvae in hatcheries is high; therefore fish hatcheries do not often achieve high production. Thus, special care has to be taken to ensure that the environment and nutritional conditions are optimal for the fish larvae.

Due to the increasing demand for food protein and the continuing acceptance of the consumers, there was an increased in the consumption of this fish. However, a sudden decreased on the production of silver barb was observed in the year 1992-2000 (Kechik, 1995; FAO, 2002). During those years, research on the larval rearing of this fish is limited and this maybe the major reason for the reduction of fish produced. This phenomenon also prompted researchers to study the techniques and methods in fish larval rearing.

The culture of *Barbodes gonionotus* had increased due to the increasing demand for protein food. One of the methods used to determine the specific nutritional requirements of the fish larvae is through the use of artificial diets comprising different levels of protein, lipids and other nutrient components.

With the increasing interest in the culture of this species, there is an urgent need for reliable, effective and efficient hatchery techniques to obtain a high and constant production and to provide the aquaculture industry with effective feeding strategies. With regards to this, research efforts must focus mainly on the formulation of appropriate feeding procedures, optimization and development of larval diet. The development of an artificial diet may constitute a cost effective alternative for the labor-intensive production of live food. The diet can be stored for a certain period of time and can be formulated to meet the specific nutritional requirements of the growing larvae. It is hypothesized that the development of an artificial diet for *Barbodes gonionotus* larvae may help in order to increase the production of this fish by reducing the mortality rate during the larval stages and to reduce the production cost.