UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT OF ARTIFICIAL DIET AND OPTIMUM FEEDING STRATEGY FOR MALAYSIAN RIVER CATFISH MYSTUS NEMURUS (CUVIER AND VALENCIENNES) LARVAE

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By

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LIST OF ABBREVIATIONS

ANOVA = Analysis of Variance

AOAC = Association of Official Analytical Chemist

Ave = Average

BSA = Bovine serum albumin

C&V = Cuvier and Valencinnes

$CHCL_3$ = Chloroform

CH_3OH = Methanol

DW-A = Direct Weaning -A

DW-B = Direct Weaning- B

EAA ratio = Essential Amino Acid ratio

EAAI = Essential Amino Acid Index

GE = Grosss Energy

GR = Growth rate

GW-A = Gradual weaning-A

GW-B = Gradual weaning-B

HPLC = High Performance Liquid Chromatography

HUFA = Highly Unsaturated Fatty acids

Kcal = Kilocalorie

Mg/Kcal = Milligrams per kilocalorie

MSA = Methano sulfonic acid

Minmix = Mineral Premix

N = Normality

Nm = nanometer

NRC = National Research Council

OPA = *O*-phtaldehyde

P:E = Protein to Energy ratio

RGR = Relative Growth Rate

RPM = revolution per minute

SGR = Specific Growth Rate

THF = Tetrahydrofuran

TRMT = Treatment

UPM = University Putra Malaysia

UV = Ultra violet

Vitmix = Vitamin premix

Abstract of the thesis submitted to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science.

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September 1998

Chairman : Dr. Mohd. Salleh Kamarudin

Faculty : Agriculture

Four feeding experiments were conducted to evaluate the growth and survival of *Mystus nemurus* larvae under different diets and feeding regimes. First experiment evaluated the influence of the gradual and direct weaning techniques on growth and survival of the larvae. Newly hatched live food (*Artemia* nauplii) was provided to all treatments at the start of exogenous feeding. For gradual weaning scheme, the live food was gradually decreased on day 4 or 6 until the larvae were on 100% artificial diet. In contrast the larval diet was shifted from live food to 100% artificial diet starting day 6 or 10 for direct weaning scheme while the controls were fed on

Artemia throughout the study. This study showed that gradual weaning at day 4 of exogenous feeding gave better growth and survival rate comparable to the controls and seemed to be the most economically and biologically viable techniques for feeding *M. nemurus* larvae.

The second experiment was conducted to study the effect of feed particle size on feeding behavior and ultimately growth and survival of larvae. Different feed particle sizes were administered at different stages of weaning. In Treatment I, the larvae were given an assorted size (<425-700µm) diet at the start of weaning onwards. Larvae on Treatment II were given different sizes of diet for different period. i.e. <425µm at the start of weaning (day 4) to day 8, 425-600µm from day 9 to day 12 and 600-700µm from day 13 to day 16. The two treatments were also tested without any weaning or live food while the control was similar to Experiment 1. Treatment I gave the best growth and survival rate equivalent to the control. Continuos feeding of a wide range of feed particle size during larviculture seemed to benefit the uneven growth rate in *M. nemurus* larvae. Treatments without weaning gave inferior growth and total mortality after day 8.

Based on the two earlier experiments, a third experiment was conducted to determine the protein requirements of *Mystus nemurus* larvae. Four isocaloric diet (4200kcal/kg) with different protein levels but with amino acid profiles similar to that of a 2-day old larvae were tested. A diet containing 60% protein level resulted in the best growth and survival during 16 day feeding trial. Which were similar to those larvae that were fed on live food.

In the last experiment, six diets with 2 protein levels (55% and 60%) and 3 energy levels (4400, 4600 and 4800mg/kcal/kg) were formulated and tested to identify the optimum P:E ratio for *M. nemurus* larvae. For the control, larvae were fed *Artemia* nauplii throughout the 16 day feeding trial. A diet containing 55% protein, 4400mg/kcal energy and P:E ratio 125mg/kcal gave significantly higher ($P>0.05$) growth and survival compared to other treatments. The performance was similar to the control. Apparent protein sparing effect may have taken place as indicated by higher growth of larvae on lower protein and higher energy level. Negative effect on growth and survival was also demonstrated when P:E ratio was below or exceeds the apparent optimum level.

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

PERKEMBANGAN DIET BUATAN LARVA DAN STRATEGI MEMBERI MAKAN YANG OPTIMA UNTUK IKAN DEDURI SUNGAI MALAYSIA, *MYSTUS NEMURUS* (CUVIER AND VALENCIENNES) LARVA.

Oleh

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Empat kejian pemakanan telah dijalankan untuk menilai tumbesaran dan kemandirian larva *Mystus nemurus* dengan diet-diet dan kaedah pemberian makanan yang berbeza. Kejian pertama menilai pengaruh teknik-teknik pertukaran makanan secara beransur dan terus ke atas tumbesaran dan kemandirian larva. Makanan hidup (naupli *Artermia*) yang baru menetas diberikan dalam semua rawatan pada permulaan pemakanan eksogenus. Untuk skim pertukaran makanan secara beransuran, makanan hidup dikurangkan secara beransuran pada hari ke-4 atau 6 sehingga larva diberi pada 100% diet tiruan. Sebagai perbandingan, diet larval ditukarkan dari makanan hidup kepada 100% diet tiruan bermula pada hari ke-6 atau 10 makanan secara terus sementara kawalan diberikan *Artemia* di sepanjang masa kajian. Kajian ini menunjukkan bahawa pertukaran makanan secara beransuran pada

hari ke-4 pemakanan eksogenus memberikan kadar tumbesaran dan kemandirian yang lebih baik berbanding dengan kawalan dan didapati sebagai teknik yang paling ekonomikal dan berkesan secara biologikal untuk pemakanan larvae *M. nemurus*.

Eksperimen kedua dijalankan untuk mengkaji kesan saiz zarah makanan ke atas tabiat pemakanan dan keseluruhannya tumbesaran dan kemandirian larva. Saiz partikel makanan yang berbeza diberikan pada peringkat-peringkat pertukaran makanan yang berbeza. Pada Rawatan I, larva diberikan diet dengan berbagai saiz (<425-700μm) pada permulaan pertukaran makanan dan seterusnya. Larvae dalam Rawatan II diberi saiz diet yang berbeza untuk jangkamasa yang berbeza. Contohnya <425μm pada permulaan pertukaran makanan (hari ke-4) sehingga hari ke-8, 425-600μm dari hari ke-9 sehingga hari ke-12 dan 600-700μm dari hari ke-13 sehingga hari ke-16. Kedua-dua rawatan turut diuji tanpa sebarang pertukaran makanan atau makanan hidup, sementara kawalan adalah seperti Eksperimen I. Rawatan I memberikan kadar tumbesaran dan kemandirian yang terbaik setara dengan kawalan. Pemberian makanan secara berterusan dengan julat saiz partikel yang besar semasa larvikultur seakan memberi kebaikan kepada kadar tumbesaran yang tidak sekata pada larvae *M. nemurus*. Rawatan tanpa pertukaran makanan memberikan tumbesaran yang rendah dan 100% kemortalan selepas hari ke-8.

Berdasarkan kedua-dua eksperimen awal, eksperimen ketiga dijalankan untuk menentukan keperluan protein larva *M. nemurus*. Empat diet isokalorik (4200kcal/kg) dengan takat protein yang berbeza tetapi dengan profil asid amino yang sama dan profil amino asid larva berumur 2 hari telah diuji. Diet yang mengandungi 60% protein menghasilkan tumbesaran dan kemandirian yang terbaik selepas 16 hari ujian pemakanan. Dan sama seperti keputusan pada larva yang diberi makanan hidup.

Di dalam eksperimen yang terakhir, enam diet dengan 2 takat protein (55% dan 60%) dan 3 takat tenaga (4400, 4600 dan 4800mg/kcal/kg) telah dihasilkan dan diuji untuk mengenalpasti nisbah P:E yang optimum untuk larva *M. nemurus*. Pada kawalan, larvae diberi makan nauplii *Artemia* di sepanjang 16 hari ujian pemakanan. Diet yang mengandungi 55% protein, 4400mg/kcal tenaga dan nisbah P:E 125mg/kcal jelas memberikan tumbesaran dan kemandirian yang lebih tinggi (P>0.05) berbanding dengan rawatan-rawatan yang lain. Penghasilan tersebut adalah setara seperti pada kawalan. Kesan penggantian penggunaan protein yang ketara mungkin telah berlaku berdasarkan yang ditunjukkan dengan tumbesaran larva yang lebih tinggi pada takat protein rendah dan tenaga tinggi. Kesan negatif ke atas tumbesaran dan kemandirian turut ditunjukkan apabila nisbah P:E adalah di bawah atau melebihi takat optimum yang aparen.

CHAPTER I

INTRODUCTION

Background of the Study

River catfish or locally known as "ikan baung" is fast gaining economic importance in Malaysian aquaculture. This species is cultured commercially and is well accepted by Malaysian consumers belonging to diverse ethnic backgrounds (Khan et al., 1990). However, inadequate seed supply coupled with relatively high fingerling prices limit its production, even though artificial breeding or reproduction of *Mystus nemurus* is routinely done at the Freshwater Aquaculture Research Center (D. O. F.) in Malacca, Universiti Putra Malaysia Fish Hatchery, as well as in other private hatcheries around Peninsular Malaysia almost all year round (Khan et al., 1990). Large-scale rearing of *M. nemurus* larvae has yet to be refined in terms of husbandry techniques and nutritional requirements of the larvae. These factors have become the major constraint to the flourishing river catfish industry in the country.

Statement of the Problem

At present, fish breeders depend mainly on live food such as *Artemia* nauplii (Johnson and Katavic, 1986; Rottman et al., 1991; Biedenbach et al., 1989; Verreth et al., 1993) for nursing fish larvae. The production of sufficient amount of live foods needs large investment on facilities, maintenance, and labor (Johnson and Katavic, 1986; Biedenbach et al., 1989; Hayashi, 1995). Live food also varies considerably in nutritional quality. This is due mainly to factors like culture condition, age and sources of stock. Varying nutritional quality greatly affects larval quality (Jones et al., 1993; Muir and Sutton, 1994). Problems associated with the routine use of natural food could hamper successful large scale hatchery operations.

According to Hayashi (1995), 67 - 88% of the live food used in the production of red seabream and Japanese flounder fry can be successfully substituted with artificial diet. Ehrlich et al. (1989) recommended that *Artemia* be initially used mainly to stabilize feeding. This can then be followed by weaning to an acceptable diet. It has been observed that when given in small amounts, zooplankton can be used as an essential component of food (Szlaminska and Pryzbyl, 1986; Verreth and Den Biemen, 1987a). It is believed that planktonic animals such as *Artemia* contain substances that are necessary for the development and growth of larvae (Fluchter 1982).

Weaning larvae from live food to artificial formulated diet has limited success because in most cases, the available feed formulations could not support the growth of the larvae prior to the onset of metamorphosis (Appelbaum, 1985). Mortality and poor growth in weaned fish may also be due to the loss of nutritional value of food due to leaching of water soluble nutrients from food particles during feeding (Tacon et al., 1986, Lee et al., 1996). Poor growth can also be attributable to the poor digestive capacity of the larvae due to an underdeveloped digestive tract (Kaushik and Luquet, 1993). These problems have generated a great amount of practical research work on not only nutrition, but also on the technology of feed manufacturing and fish physiology (Verreth et al., 1987b; Cuzon et al., 1994).

One must first determine the basic feeding requirements (diet composition, form, feeding frequency and rate) before appropriate artificial feeds are formulated and feeding trials are conducted. Factors like egg quality, diet acceptability, rearing environment and other husbandry skills must be considered to ensure the success of feeding experiments in larval fish (Rust et al., 1993). The appropriate stage when the fish can readily accept artificial diet must also be investigated. Transition from live food to artificial diet depends on the quality of feed and the larvae itself (Devresse et al., 1991). Once larval feeding has commenced, full development of appetite, physiology, behavior and learning to accept artificial diet exert major influences on the choice of food and the readiness to feed (Knights, 1996). Several approaches in weaning *M. nemurus* larvae should be tested. Moreover, the scheme that best suits the larval requirements and the limited investment capacity of the fish breeders must be adopted.

Numerous factors influence the success of weaning These include particle size, composition and feeding rate, and the quality and quantity of the feed if larvae are to be weaned very early (Bromley, 1978). Food given in amounts which permit maximal growth and minimum wastage is also an important consideration (Bryant and Matty, 1981).

Hogendoorn (1980) concluded that the inadequate performance of fry on artificial diet is caused by poor utilization. Learning to accept artificial diet appears to be the dominant factor in food selectivity. However, it is important to use feeds with suitable dimension and texture to optimize consumption and help maintain good water quality (Knights, 1983). Feeding of *M. nemurus* is believed to be strongly influenced by food particle size in relation to the mouth size of the larvae. Hence, particle size should be evaluated first prior to every experiment in order to determine optimum feeding rate.

The need for a suitable artificial diet for *M. nemurus* larvae to partially or totally replace live food is very important. This will increased production of marketable-sized fish by ensuring sustainable and adequate supply of juveniles. It is envisioned that this study will pave the way towards the development of an appropriate artificial diet for *M. nemurus* larvae. It is hoped that details regarding feed size, form and composition, and the right time at which artificial feeds can be given will be defined.

General Objective of the Study

To identify the optimum feeding strategy that will ensure maximum utilization of food during larval stage of *Mystus nemurus*, and to develop a practical diet that will partially or totally reduce the dependence of *M. nemurus* to live food during larval rearing.

CHAPTER II

LITERATURE REVIEW

Live Prey as Larval Food

In any potential fish species for aquaculture, problems may be encountered from the time larval rearing and nutrition begin to the time fish are harvested. At present, the general principle of starting larval rearing with the use of live food in larviculture is being employed since this is the stage when the physiological capabilities of larvae are believed to be limited.

The natural diet of most aquaculture species (fish and crustaceans) consists of a wide range of phytoplankton and zooplankton species. In aquaculture, total dependence on plankton from the wild for use as larval food is not a reliable nor a commercially feasible strategy (Sorgeloos and Leger, 1992). The production of even the most suitable plankton species still poses many problems (e.g. culture maintenance and contamination) in large scale culture (Katavic, 1986; Biedenbach, 1989; Rottman et al., 1991; Hayashi, 1995). Due to their relatively small size, rotifers are used as starter diets in marine fish larviculture. However, many fish hatcheries experience considerable problems as far as maintaining large cultures and producing enough supply of rotifers are concerned (Sorgeloos and Legger, 1992; Holt, 1992; Jones et al., 1993). Some authors have confirmed that producing sufficient amounts of live diet requires too much investment for facilities, maintenance and labor (Biedenbach et al., 1989; Katavic, 1984; Kanazawa et al., 1998; Hayashi, 1995). Another type of live diet popularly used in larviculture is the brine shrimp, *Artemia salina* (Watanabe et al., 1983; Rottman et al., 1991; Sorgeloos and Leger; 1992, Jones et al., 1993). *Artemia* nauplii, which can be hatched from commercially and readily available cysts, apparently contain most of the essential micro and macro nutrients for the larvae (Pector et al., 1994). However, several strains of *Artemia*, including some commercial ones, produced very inconsistent results on larval fish growth and survival (Navarro et al., 1988). There are several factors that influence the efficiency of *Artemia* as larval food. One of these is naupliar size which varies from one geographical source to another. Naupliar size is critical for several species of fish larvae particularly those that have very small mouth size. Fish that swallow their prey on one bite cannot take *Artemia* due to its slightly bigger size than rotifers (Sorgeloos and Legger, 1992; Muir and Sotton, 1994). Some investigators have tried *Artemia* as a non-living diet in the form of decapsulated cysts to minimize size and production-related problems (Pector et al., 1994).

Several authors have studied the potential of enriching live food prior to feeding them to larval fish (Watanabe et al., 1983; Walford et al., 1987; Strottup and Attramal, 1992; Dhert et al., 1992; Kraul et al., 1993; Tandler, 1993; Jones et al. 1993). Rotifers and *Artemia* have been demonstrated to be capable of incorporating n-3 HUFA when supplied to live prey in the form of microencapsulated foods. However, this process demands additional inputs on the part of hatchery operator.

The feeding of live food for intensive larval rearing generally comprises a considerable percentage in the production cost of fish and crustacean juveniles. Minimizing the use of live food for larviculture will reduce this cost. However, two decades of research on the formulation of microdiets to replace live food in larviculture have resulted to limited success (Dabrowski, 1984; Henken et al., 1986; Verreth et al., 1987b; Rust et al., 1993; Watanabe et al., 1994; Cuzon et al., 1994). To reduce the use of live food, hatchery operators supply fish with live diet in combination with an adequately formulated diet. This makes early weaning to artificial diet easier (Watanabe and Kiron, 1994). Le Ruyet et al. (1993) estimated that if weaning of marine fish such as seabass or turbot starts at day 25 instead of day 35, the expected live prey savings would be as high as 60%. If weaning starts 5 days earlier, that is at day 20, the expected live prey savings is 80%. Ehrlich et al. (1989) and Bryant and Matty (1981) stressed that *Artemia* should be used only to stabilize feeding following weaning to an acceptable diet. Szlaminska and Pryzbyl (1986) assumed that zooplankton given in small amounts may be an essential component of food because it may contain substances necessary for the development and growth of larvae. On the other hand, Verreth et al. (1993) observed that the best growth in feeding larval catfish *Clarias gariepinus* is obtained when ample amounts of live food are administered during day 1 of exogenous feeding.