UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DIETARY PROTEIN AND PROTEIN ENERGY RATIO ON GROWTH PERFORMANCE OF LEMON FIN BARB HYBRID (Hypsibarbus wetmorei X Puntius gonionotus) LARVAE

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Chairman: **Assoc. Prof. Che Roos b Saad, PhD**

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This study was carried out to determine basic requirement of larval Lemon Fin Barb hybrid. There were three experiments in this study. The first experiment is considered as preliminary experiment. The objective is to measure the mouth gape of the larvae. This was done in order to get the right particle size of formulated diet that will be utilized in experiment protein and protein energy ratio requirement of the larvae. The newly hatched larvae were collected from Aquaculture Extension Research Center, Perlok, Pahang, Malaysia. The feeding trial was done in twenty-one days by feeding the larvae with frozen crushed *Artemia* due to its small mouth gape size than size of *Artemia* nauplii which ranged from two hundred to four hundred micron. The mouth of larvae opened at the third day after hatched with 49 micrometer mouth gape at 45 degrees. Size of frozen crushed *Artemia* was 40.93 ± 4.12 micrometer. This size fit well with the mouth gape size of the larvae since day three after hatched. By referring to results obtained in mouth gape study, in subsequent protein and protein energy ratio studies, formulated diet was sieved with 50 micrometer sieve on third day after hatched, on thirteen day after hatched formulated diet was sieved with 100 micrometer sieve and on seventeen day after hatched onwards formulated diet was sieved with 150 micrometer sieve.

A series of two experiments were conducted to study the optimum dietary protein level and protein to energy ratio of Lemon Fin Barb hybrid larvae. In experiment one, five formulated diets (4700 kilocalorie per kilogram) ranging from forty to sixty percents protein in five percents increments were fed for twenty-one days to triplicate groups of Lemon Fin Barb hybrid larvae (initial weight: 1.62 ± 0.03 miligram per fish). Larvae were weaned into formulated diet on day six after hatched. From day three to day five after hatched larvae were fed with frozen crushed *Artemia* in order to develop gustatory response to the digestive system of the larvae. Sampling was done at four days interval. Data on weight, length and survival were taken during sampling process. Weight gain of fish was proportional to the protein content of the diet up to an incorporation rate of

fifty percents. Among formulated diets, diet with fifty percents protein recorded the highest weight gain (39.03 ± 0.71 miligram). The dietary protein level that yielded maximum growth was forty-nine percents based on a broken-line model estimation of weight gain. Due to this result, two dietary protein levels (45 percents and 50 percents) were used along with four energy levels at each protein level (4500, 4700, 4900 and 5100 kilocalorie per kilogram diet) in experiment two.

In experiment two, eight diets were formulated consisting of protein level (45 percents and 50 percents) with energy level (4500, 4700, 4900 and 5100 kilocalorie per kilogram diet). The survival of fish fed diets containing 45 percents protein was significantly lower ($P<0.05$) than that of fish fed diets containing 50 percents protein regardless of energy level. Weight gain of fish was significantly ($P<0.05$) different at all dietary protein and energy levels. The best growth was observed in larvae fed diet 7 containing 50 percents protein with 4900 kilocalorie per kilogram energy with the highest weight gain (43.80 ± 0.64 miligram), best feed conversion ratio (1.05 ± 0.04) and protein energy ratio of 99.36 miligram per kilocalorie.

In conclusion Lemon Fin Barb hybrid larvae started opening mouth on third day after hatched at 49 micrometer mouth gape at 45 degrees. The optimum protein level in diet for this new hybrid is fifty percents while the optimum energy is 4900 kilocalorie per kilogram with protein energy ratio of 99.36 miligram per kilocalorie.

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

KESAN DIET PROTIN SERTA NISBAH PROTIN DAN TENAGA TERHADAP PERTUMBUHAN LARVA HIBRID KERAI LAMPAM (*Hypsibarbus wetmorei* X *Puntius gonionotus*)

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Pengerusi: Prof. Madya. Che Roos b Saad, PhD

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Kajian ini dijalankan untuk menentukan keperluan asas larva ikan kacukan Kerai Lampam. Terdapat tiga eksperimen dalam kajian ini. Eksperimen pertama dikategorikan sebagai eksperimen pendahuluan. Objektifnya adalah untuk mengukur bukaan mulut larva ini. Ini dilakukan bagi mendapatkan saiz yang tepat untuk membuat makanan buatan yang akan digunakan dalam eksperimen protin dan nisbah protin tenaga larva. Larva yang baru menetas diambil dari Pusat Perkembangan Penyelidikan Akuakultur, Perlok, Pahang, Malaysia. Ujian pemakanan dijalankan selama dua puluh satu hari dengan memberi larva makan *Artemia* beku yang telah dihancurkan berikutan saiz bukaan mulut yang kecil jika dibandingkan saiz *Artemia* naupli yang berukuran antara dua ratus ke empat ratus mikron. Mulut larva mulai terbuka pada hari ketiga selepas menetas dengan saiz 49 mikrometer bukaan pada sudut 45 darjah. Saiz *Artemia* beku yang telah dihancurkan adalah 40.93 ± 4.12 mikrometer. Saiz ini sepadan dengan bukaan mulut larva sejak pada hari ketiga ia menetas. Dengan merujuk kepada keputusan yang diperolehi dalam kajian bukaan mulut larva, dalam kajian keperluan protin dan protin tenaga, makanan buatan ditapis dengan tapis berukuran 50 mikrometer pada hari ketiga selepas menetas, pada hari ke 13 selepas menetas makanan buatan ditapis dengan 100 mikrometer saiz tapisan dan pada hari ke 17 dan seterusnya, makanan buatan ditapis dengan tapisan berukuran 150 mikrometer.

Satu siri kajian yang mengandungi dua eksperimen telah dijalankan bagi menentukan kadar keperluan optima protin dan nisbah protin tenaga dalam larva ikan kacukan Kerai Lampam. Dalam eksperimen pertama, lima makanan buatan (4700 kilokalori per kilogram tenaga) berjulat dari 40 ke 60 peratus protin dengan pertambahan 5 peratus telah diberi selama 21 hari kepada tiga replikasi kumpulan larva kacukan Kerai Lampam (berat awal: 1.62 ± 0.03 miligram per ikan). Pemakanan larva ditukar ke makanan buatan pada hari keenam selepas menetas. Dari hari ketiga hingga hari kelima selepas menetas, larva diberi makan *Artemia* beku yang telah dihancurkan dengan tujuan untuk membina respon gustatori kepada sistem penghadaman larva.

Pengambilan sampel dilakukan selang empat hari. Data berat, panjang dan kebolehan hidup diambil sewaktu proses pengambilan sampel. Pertambahan berat badan ikan didapati selari dengan pertambahan nilai protin ke tahap pertambahan 50 peratus protin. Antara makanan buatan, makanan yang mengandungi 50 peratus protin mencatatkan kadar pertambahan berat badan yang paling tinggi (39.03 ± 0.71 miligram). Kadar protin yang menghasilkan kadar pertumbuhan tertinggi adalah 49 peratus berdasarkan model perkiraan titik-putus dalam pertambahan berat badan. Berdasarkan keputusan ini, dua kadar protin (45 peratus dan 50 peratus) digunakan bersama empat kadar tenaga pada setiap kadar protin (4500, 4700, 4900 dan 5100 kilokalori per kilogram makanan) dalam eksperimen kedua.

Dalam eksperimen kedua, lapan makanan buatan dibuat yang terdiri daripada kadar protin (45 peratus dan 50 peratus) digunakan bersama empat kadar tenaga (4500, 4700, 4900 dan 5100 kilokalori per kilogram makanan). Kadar kebolehan hidup ikan dengan makanan mengandungi 45 peratus protin didapati lebih signifikan rendah ($P<0.05$) berbanding ikan dengan makanan mengandungi 50 peratus protin tanpa mengira kadar tenaga. Kadar pertambahan berat badan ikan adalah signifikan berbeza ($P<0.05$) dalam semua kadar protin dan tenaga. Kadar pertumbuhan terbaik didapati dalam larva yang makan makanan ketujuh yang mengandungi 50 peratus protin bersama 4900 kcal per kg tenaga dengan pertambahan berat badan (43.80 ± 0.64 miligram), nisbah penukaran makanan terbaik (1.05 ± 0.04) dan nisbah protin tenaga 99.36 miligram per kilokalori.

Secara keseluruhannya larva ikan kacukan Kerai Lampam mula membuka mulut pada hari ketiga selepas menetas dengan bukaan 49 mikrometer pada sudut 45 darjah. Kadar optima protin dalm diet bagi larva hibrid baru ini adalah lima puluh peratus manakala kadar optima tenaga adalah 4900 kilokalori per kilogram bersama nisbah protin tenaga 99.36 miligram per kilokalori.

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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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LIST OF ABBREVIATIONS

AAAA	apparent amino acid availability
AAs	amino acids
ADGl	average daily length gain
APD	apparent protein digestibility
CH	Chironomids
CHFD	Chironomids + formulated diet
Cl^-	chloride ion
DAH	day after hatch
DE	digestible energy
DL	dietary lipid
DP	Daphina
DP	dietary protein
DP/DE	digestible protein to digestible energy ratios
DPFD	Daphina + formulated diet
dph	day post-hatch
E/P	Energy/Protein ratios
ER	energy retention
FCR	Feed conversion ratio
G	Gammarid
GE	gross energy
GFD	Gammarid + formulated diet
HP	heat production
HUFA	highly unsaturated fatty acids

kJ	kilojoule
MBD	microbound diet
ME	metabolizable energy
N	nitrogen
Na^+	sodium ion
NE_g	net energy used for gain
NEg	net energy for gain
NE_l	net energy used for lactation
NE_m	net energy used for maintenance
NFE	Nitrogen Free Extract
PER	protein efficiency
PER	protein utilization efficiency
PPV	protein retention
PUFA	polyunsaturated fatty acids
S.D.	Standard deviation
SD	standard deviation
SGRl	specific growth rate in length
SGRw	Specific growth rates in weight
TAAA	true values amino acid availability
TDN	total digestible nutrients
TL	total length
TPD	true values protein digestibility
UJ	upper jaw
VFI	maximum voluntary feed intake
ω-3	omega 3

ω-6	omega 6

CHAPTER 1

INTRODUCTION

Lemon Fin Barb hybrid fish has been successfully produced by the cross breeding using male Kerai Kunyit, *Hypsibarbus wetmorei* and female Lampam Jawa, *Puntius gonionotus*. Lemon Fin Barb fish possessing desirable taste and high market price. The price could achieve around 42 to 48 MYR (Malaysian Ringgit) per kilogram in Malaysia. Its high price and market demand has triggered more interest in culturing this new hybrid under more intensive conditions. Moreover, this new hybrid could be marketed as fermented fish, smoked fish and salted fish (Mr. Hatta, personal communication). Little is known about their nutritional requirements as it is the newest hybrid in Malaysia. To our knowledge, data and information regarding optimal dietary protein energy ratio for Lemon Fin Barb hybrid have not been published. According to Sung and Kyeong (2009), nutritional requirements for each species is very crucial to be determined as this could improve growth of species and reducing its culture period as well as decreasing feed cost.

According to Kechik (1995), in Malaysia, Javanese carp *Puntius gonionotus* was one of species cultured in cages. Unfortunately information on this Lemon Fin Barb hybrid species is too limited and scarce. The knowledge of nutritional requirements in Lemon Fin Barb hybrid species is essential to improve the productive development but most rearers do not know scientifically nutritional requirements for this hybrid. As the nutritional information on this new hybrid is too limited and scarce, these studies were conducted to fill the emptiness. In spite of the importance of this sector in economic view, the nutritional information for Lemon Fin Barb hybrid species is scarce or even no data of the nutritional requirements is accessible.

As protein is the most expensive part in fish feed, it is crucial to determine protein requirements accurately for each species and size of cultured fish. Protein and amino acid requirements of each fish species reared is important to be acknowledged and matched (Craig and Helfrich, 2009). Generally amino acid profile of fish body is a good pointer of its requirements of that fish (Mehmat, 2008).

Energy is a crucial part of dietary and is considered as a basal component of food and to maintain body growth. However, the most important part of the diet and act as the main cost is protein (Ghiasvand *et al*., 2012). Energy and protein dietary nutrients are

vital for the building of living tissues. They could be a source of stored energy for fish digestion, absorption, growth, reproduction and the other life processes (Craig and Helfrich., 2009). In the case of abalone, Gomez-Montes *et al*. (2003) stated in order to reach maximum growth, protein deposition must be maximized and formulated diets must comprise a proper balance of appropriate sources of protein and energy.

To ensure stability in growth and survival of the *Barbonymus gonionotus* fry, Ahammad *et al*. (2009) suggested that effect of various factors such as appropriate feeding and feeding frequency must be properly understood and accordingly managed. In order to increase larval fish production efficiency and eliminating too much dependance on live diets, improved diet formulation and manufacturing technologies should be foreseen (Barrows and Lellis, 2006). Therefore, artificial diets for Lemon Fin Barb hybrid larvae were formulated and tested in these studies.

This research was done in order to investigate on the basic protein energy ratio requirements of Lemon Fin Barb hybrid larvae.

1.1 Justification

D'Abramo (2002) reported that industry of culturing larvae of many species of fish and crustaceans is too much depending on live food. Even though *Artemia* and rotifers could serve as excellent sources, users should realize that live *Artemia* nauplii are obtained through cysts that are collected from natural environment and this may subject to periodic, unpredictable shortages that cannot always supply the demand. The effect of this phenomenon is increment in prices and might be expected to lead to higher production costs. Temporal differences in cyst collections resulted in variation in the nutritional quality of *Artemia*. Moreover, disadvantages of depending entirely on *Artemia* as feed will make hatchery operations highly cost and unsustainable. Thus in this study, formulated diets were utilized to substitute live food as alternative for acceptable diets.

There is deficiency in studies relative to energy requirements in spite of the importance of protein/energy, protein/digestible energy and protein to lipid ratio (P/L) in fish nutrition (Robaina and Izquierdo, 2000). The aim of this research is to appraise the specific nutritional requirements of Lemon Fin Barb hybrid larvae which are prerequisite to improve economical and productive potential of this freshwater fish. Optimum protein and protein energy ratio in feed for this species will be determined.

1.2 Objectives

(i) To examine larval mouth gape and making appropriate size of food particle.

(ii) To determine the optimum level of protein requirement of Lemon Fin Barb hybrid larvae.

(iii) To investigate the optimum level of protein to energy ratio of Lemon Fin Barb hybrid larvae.

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