ROLE OF DIETARY LIPID IN GROWTH AND DISEASE RESISTANCE OF HYBRID LEMON FIN BARB LARVAE (*Hypsibarbus wetmorei* SMITH 1931 ♂×*Barbonymus gonionotus* BLEEKER 1849 ♀)

By

WEERASINGHA ROCHANA

Thesis Submitted to the School of Graduate Studies, University Putra Malaysia in Fulfillment of the Requirement for the Degree of Doctor of Philosophy

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DEDICATION

To my beloved family, firstly my late father (Mangala Weerasingha), my mother (Somalatha Malani Weerasingha) for strengthening me in all ways with unconditional love and care, secondly my wife (Mayuri Prasadika Hengedara) and my kids (Methira Manhiru Weerasingha and Rithara Risindi Weerasingha) for the immense support and patience paid until I come to the endpoint.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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WEERASINGHA ROCHANA

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Chairman : Professor Mohd Salleh Bin Kamarudin, PhD
Faculty : Agriculture

Most carp larvae including hybrid lemon fin barb readily accept formulated microdiets at first feeding. Dietary protein and energy requirements for this larval hybrid, a cross of *Hypsibarbus wetmorei* male and *Barbonymus gonionotus* female, have been reported. This study focused on determining its lipid requirements including the best lipid source and the requirements for phospholipids and cholesterol. In Experiment 1, three days old larvae were randomly stocked at 10 larvae L^{-1} in fifteen 9 L tanks and fed the test diets containing 0, 4, 8, 12 and 16 % cod liver oil for 20 days four times a day. The larvae fed elevated dietary lipid levels exhibited higher (p<0.05) survival and growth compared to those fed a lipid-free diet. Excessive dietary lipid levels (>13.5%) reduced the larval growth and increased lipid droplets in the liver and intestine indicating a 13.5% optimum dietary lipid level for maximum growth. In Experiment 2 and the following experiments, larvae were stocked at the rate of 50 larvae L^{-1} in fifteen 5 L tanks. Cod liver oil was replaced with crude palm oil at 0, 25, 50, 75 and 100 %. Dietary replacement of cod liver oil did not affect (p>0.05) survival and growth of hybrid larvae. Lipid vacuoles were observed in the liver and intestine of larvae fed 0, 25 and 100 % crude palm oil when challenged with *Aeromonas hydrophila*. Five plant oils were evaluated in Experiment 3. The survival of larvae fed crude palm oil was significantly higher (p<0.05) than those fed linseed oil, canola oil and soybean oil. The growth of larvae fed crude palm oil and linseed oil diets was significantly higher (p<0.05) than that of larvae fed with soybean oil. Isolipidic diets containing 0, 1, 2, 4 and 6 % soy lecithin were tested in Experiment 4. While the survival was not affected, the weight gain and protein efficiency ratio (PER) of hybrid larvae fed 4% soy lecithin were significantly higher (p<0.05) than those of larvae fed 0-2 % soy lecithin. Lipid vacuoles decreased in the liver and gut of hybrid larvae fed 2 and 4 % dietary phospholipid when challenged against *A. hydrophila*. The recommended dietary phospholipid level for the hybrid larvae was 4%. In Experiment 5, five isolipidic diets containing 0, 0.5, 1, 1.5 and 2 % cholesterol with 4% phospholipid were tested. Elevated dietary cholesterol levels did not affect the survival and growth of hybrid larvae but increased lipid vacuoles in their liver and gut. Disease resistance against *A. hydrophila* decreased with the inclusion of dietary cholesterol. In conclusion, hybrid

lemon fin barb larvae required 13.5% lipid, includes 4% phospholipid. Dietary cholesterol did not improve larval disease resistance and their survival and growth. Crude palm oil was the best plant oil that could fully replace fish oil in hybrid larval diets.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PERANAN LIPID DALAM MAKANAN TERHADAP PERTUMBUHAN DAN RINTANGAN PENYAKIT LARVA KERAI LAMPAM (*Hypsibarbus wetmorei* SMITH 1931 ♂ × *Barbonymus gonionotus* BLEEKER 1849 ♀)

Oleh

WEERASINGHA ROCHANA

Disember 2022

Pengerusi : Profesor Mohd Salleh Bin Kamarudin, PhD
Fakulti : Pertanian

Kebanyakan larva ikan kap termasuk kerai lampam sedia menerima diet mikro terumus bagi makanan pertama. Keperluan protein dan tenaga dalam diet larva kerai lampam, kacukan daripada *Hypsibarbus wetmorei* jantan dan *Barbonymus gonionotus* betina, telah dilaporkan. Kajian ini memfokus kepada penentuan keperluan lipidnya termasuk sumber lipid terbaik serta keperluan fosfolipid dan kolesterol. Dalam Eksperimen 1, larva berumur tiga hari telah distok secara rawak pada kadar 10 larva L^{-1} dalam lima belas tangki 9 L dan diberi ujian diet yang mengandungi 0, 4, 8, 12 dan 16 % minyak hati ikan kod sebanyak empat kali sehari selama 20 hari. Larva yang diberi makan lipid berparas tinggi mempamerkan kemandirian dan pertumbuhan yang lebih tinggi (p<0.05) berbanding dengan larva yang diberi diet tanpa lipid. Paras lipid yang berlebihan (>13.5%) dalam makanan mengurangkan pertumbuhan larva dan meningkatkan titisan lipid dalam hati dan usus menunjukkan paras optimum lipid dalam diet adalah 13.5% untuk pertumbuhan yang maksimum. Dalam Eksperimen 2 dan eksperimen seterusnya, larva telah distok pada kadar 50 larva L^{-1} dalam lima belas tangki 5 L. Minyak hati ikan kod telah diganti dengan minyak sawit mentah pada 0, 25, 50, 75 dan 100 %. Penggantian minyak hati ikan kod dalam makanan tidak menjejaskan (p>0.05) kemandirian dan pertumbuhan larva kerai lampam. Vakuol lipid kelihatan dalam hati dan usus pascalarva yang diberi 0, 25 dan 100 % minyak sawit mentah bila dicabar dengan *Aeromonas hydrophila*. Lima minyak tumbuhan telah dinilai dalam Eksperimen 3. Kemandirian larva yang diberi minyak sawit mentah adalah ketara lebih tinggi (p<0.05) daripada larva yang diberi minyak biji rami, minyak kanola dan minyak kacang soya. Pertumbuhan larva yang diberi minyak sawit mentah dan minyak biji rami adalah ketara lebih tinggi (p<0.05) berbanding pertumbuhan larva yang diberi minyak kacang soya. Diet isolipid yang mengandungi 0, 1, 2, 4 dan 6 % lesitin kacang soya telah diuji dalam Eksperimen 4. Walaupun kemandirian tidak terjejas, pertambahan berat badan dan nisbah kecekapan protein (PER) larva kerai lampam yang diberi 4% lesitin kacang soya adalah ketara lebih tinggi (p<0.05) dari larva yang diberi 0-2 % lesitin kacang soya. Vakuol lipid berkurangan dalam hati dan usus pascalarva kerai lampam yang diberi 2 dan 4 %

fosfolipid bila dicabar dengan *A. hydrophila*. Paras fosfolipid yang disyorkan dalam makanan larva kerai lampam ialah 4%. Dalam Eksperimen 5, lima diet isolipid mengandungi 0, 0.5, 1, 1.5 dan 2 % kolesterol dan 4% fosfolipid telah diuji. Peningkatan paras kolesterol dalam makanan tidak menjejaskan kemandirian dan pertumbuhan larva lampam kerai tetapi meningkatkan vakuol lipid dalam hati dan usus larva. Rintangan penyakit terhadap *A. hydrophila* menurun dengan kehadiran kolesterol dalam makanan. Kesimpulannya, larva kerai lampam memerlukan 13.5% lipid yang merangkumi 4% fosfolipid. Kolesterol dalam makanan tidak meningkatkan ketahanan penyakit larva dan kemandirian dan pertumbuhan mereka. Minyak sawit mentah adalah minyak tumbuhan terbaik yang boleh menggantikan minyak ikan sepenuhnya dalam diet larva kerai lampam.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Mohd Salleh bin Kamarudin, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Murni Marlina binti Karim, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Mohammad Fadhil Syukri bin Ismail, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:8 June 2023

Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- Supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

Signature: ______________________________
Name of Chairman of
Supervisory Committee: Professor Dr. Mohd Salleh bin Kamarudin

Signature: ______________________________
Name of Member of
Supervisory Committee: Associate Professor Dr. Murni Marlina binti Karim

Signature: ______________________________
Name of Member of
Supervisory Committee: Dr. Mohammad Fadhil Syukri bin Ismail

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WG	weight gain
SGR	specific growth rate
FCR	feed conversion ratio
PER	protein efficiency ratio
Min	minutes
h	hours
EFA	essential fatty acid
SFA	saturated fatty acid
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
HUFA	highly unsaturated fatty acid
LA	linoleic acid
ALA	alpha linolenic acid
ARA	arachidonic acid
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
TAG	triacylglycerol
LPL	lipoprotein lipase

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Alien carps have dominated the carp culture in Malaysia. The indigenous carps accounted for only 30.52% of the total carp production in 2020 (DOF, 2021). Indigenous carp production has also decreased from 8646.45 tonnes to 3933.28 tonnes between 2012 (DOF, 2013) and 2020 (DOF, 2021). On the other hand, lemon fin barb (*Hypsibarbus wetmorei*), locally called as 'kerai kunyit', fishery is a conventional fishery in Malaysia, especially in Pahang, Perak and Kelantan rivers and inland lakes near to those rivers. The average retail price of this barb has increased from RM 15.43 kg^{-1} in 2004 up to RM 57.43 kg^{-1} in 2020 (DOF, 2021). With the ready demand, lemon fin barb catch has been increased from 10.47 to 40.00 tonnes in between 2012 (DOF, 2013) and 2020 (DOF, 2021). The good taste of *H. wetmorei* is the main reason for this consumers' preference (Suharmili et al., 2015). According to Chong et al. (2010), freshwater fish wild stock in Malaysia are being overexploited and the habitats of freshwater fish have been degraded due to pollution, logging, development and siltation. Lemon fin barb *H. wetmorei* is grouped into least concern (LC) category in IUCN red list (Rashid et al., 2015). To efficiently control its fishing pressure, Department of Fisheries (DOF), Malaysia has crossed the (male) of this species with female silver barb, *Barbonymus gonionotus* which has a fast growth rate to produce hybrid lemon fin barb for aquaculture purposes.

The larval stage of aquatic animals represents the most significant stage or phase in the life cycle as the foundation for fish health starts from there (Zhang et al., 2018). As larvae start at a very tiny size and have a fast growth, their nutrient deficiencies are easily expressed (Dabrowski, 1984). At the first feeding, nutrient reserves in fish larvae are low hence larval growth performance is especially correlated with the provided feeds that must contain all the essential nutrients to meet their nutritional requirements (Kolkovski et al., 2009). The nutritional requirements of larvae and nutrients in diets must be considered together in the larval nutrition as they act synergistically (El-Kertaoui et al., 2019). Applying extra lipids in fish diets over the optimum level can cause undesirable consequences such as low feed consumption, high fat deposition, reduced growth performance and inefficient utilization of other nutrients (Wang et al., 2005).

High mortality is a common problem in a hatchery production which is usually a bottleneck for the expansion of the industry (Ai et al., 2008; Bricknell & Dalmo, 2005). Several biotic factors like diseases, low energy supply and abiotic factors like water quality parameters as well as physiological stress have strong effect on early mortality of juveniles (Gosselin & Qian, 1997; Hunt & Scheibling, 1997). *Aeromonas hydrophila* is known as a pathogenic bacteria that commonly cause mortalities in carps (Alsaphar & Al-faragi, 2012).

D'Abramo (2002) had reported on the high dependency of most hatcheries on live foods. Live foods like *Artemia* and rotifers can be very costly to produce, periodic or unpredictable in availability or supply, inconsistent or deficient in nutritional quality, and may carry pathogens (Takeuchi, 2014; Zhang et al., 2018). To enhance the production of postlarvae and reduce the highly dependency on live foods, the development of palatable, nutritionally balanced larval microdiets and their manufacturing technologies must be pursued (Anizah et al., 2017; Zhang et al., 2018). Fish larvae can ingest microdiets but the inadequate understanding of larval nutrition and digestive capacity, and inappropriate feeding techniques often lead to low survival and reduced growth rate (Kanazawa et al., 1989; Jones et al., 1993; Kamarudin et al., 2011). Benefits of partial replacement of live feed with microdiets at the onset of exogenous feeding in bivalve, crustacean and fish larvae have been highlighted by Jones et al. (1993). They stated that freshwater fish larvae are relatively larger at hatching and have functional stomach, and hence they can easily feed dry feed at first feeding. For instance, *Cyprinus carpio* (Charlon & Bergot, 1984; Charlon et al., 1986) and *Coregonus lavaretus* (Champigneuille, 1988) larvae have shown good growth and survival when fed artificial diets from the start of first feeding. However, the low digestive capacity of fish larvae at first feeding has been indicated as a main limiting factor in larval culture. However, the capacity of fish larvae to digest artificial diets differs from species to species.

Lipid is the second major concern after protein in larval fish nutrition for the optimization of protein digestion and utilization, and maximal growth. Lipids are important to maintain the structure of cell membrane and support to its general functions (Sargent et al., 1995) and as precursors of paracrine hormones which are known as eicosanoids (Sargent et al., 1999). Lipid is also an important component that provides energy and essential fatty acids (EFA) for the development of laevae (Ai et al., 2008). The length of FA carbon chain and nature of lipid whether saturated or unsaturated may influence lipid digestion and absorption. Fatty acid profile and dietary triacylglycerol (TAG) level in fish feeds are the most important parameters to reveal that mechanism (Austreng et al., 1979; Koven et al., 1994b; Olsen et al., 1998; Morais et al., 2004). Normally, fatty acid profile varies with lipid sources. Tocher (2010) emphasized the necessity of biochemical and molecular knowledge on essential fatty acid (EFA) requirements to solve dietary issues of larval feeds. A high level of medium chain fatty acids in common carp larvae diets reduces their growth and survival (Fontagne et al., 2000). Pozernick & Wiegand (1997) had discussed on the possibility of substituting fish oil in cyprinid feeds without affecting feed efficiency, growth and reproduction. Janaranjani et al. (2018) suggested the use of vegetable oil in carp farming. In general, C_{18}-poly unsaturated fatty acids (PUFA), especially linoleic acid (LA) and α-linolenic acid (ALA), fulfill the EFA requirement of most freshwater fish (Tocher, 2010). Besides, a satisfactory dietary lipid level in diets improves the synthesis of lipoprotein lipase (LPL) and the activity of fatty acid synthetase (FAS) that are necessary for lipid digestion (Zheng et al., 2010; Zhang et al., 2018). Lipids can be completely digested in the fish gut (Cowey & Sargent, 1977) though lipid digestion rate is very low in fish larvae (Olsen & Ringø, 1997). Larvae cannot digest large amount of lipid (Olsen & Ringø, 1997). In addition to weak digestive enzyme activity, a fish larva has a short and simple digestive tract. Kiron et al. (2004) highlighted several benefits of using different lipid sources like good immune response, antioxidative functions and other physiological functions. Recently, many researchers showed interests in larval nutrition with special attention on digestion, absorption, caring and metabolic process of lipid. Izquierdo et al. (2000) noted that the lipid absorption capacity is increased in the larvae fed live prey while its absorption is delayed when larvae are fed a compound feed. Dietary lipids have another role in sparing dietary protein that can lower down organic substances, and nitrogen and energy losses (Cho and Kaushik, 1990). However, a proper dietary protein to lipid ratio is critical for effective nutrient utilization as it significantly affects growth and immune response (Chen et al., 2012) and improves lysozyme and digestive enzyme activities (Cheng et al., 2006) in aquatic creatures. Sheen & Wu (1999) stated that the fatty acid levels in the feed must also be essentially evaluated while finding out the optimum dietary lipid requirement level. Moreover, Izquierdo et al. (2000) highlighted that the dietary ratios of essential fatty acids must also be considered for the optimum dietary supply of individual EFAs for fish larvae.

1.2 Problem Statement

Sustaining the aquaculture of hybrid lemon fin barb is quite challenging due to the lack of proper feeds for its larval culture (Anizah et al., 2017) while not many hatcheries are capable of producing live foods. The number of hatcheries that can produce hybrid lemon fin barb fry remains low (DOF, 2021).

The influences of lipid on growth and disease resistance in juvenile and adult fish have been discussed a lot (Huang et al., 2015) while only a few studies had been focused on lipid nutrition in larval fish (Zheng et al., 2010; Zhang et al., 2018). However, the optimum dietary lipid requirement, suitable lipid substitutes for maximum growth and survival; and disease resistance against *Aeromonas hydrophila* and the effects of dietary phospholipids supplementation must be investigated for larval hybrid lemon fin barb and is of a paramount important for its sustainable fry production.

1.3 Objectives of the study

The general objective of the study was to evaluate dietary lipid requirement on growth and disease resistance of hybrid lemon fin barb (*Hypsibarbus wetmorei* ♂ × *Barbonymus gonionotus* ♀) larvae while the specific objectives were:

1. To determine the dietary lipid requirement of larval hybrid lemon fin barb
2. To examine fish oil replacement with crude palm oil on growth, survival, enzyme activity and disease resistance of hybrid lemon fin barb larvae
3. To investigate the effects of various plant-based oil sources on the survival, growth and body composition of larval hybrid lemon fin barb
4. To examine the effects of dietary soybean lecithin level on growth, survival, enzyme activity and disease resistance of larval hybrid lemon fin barb
5. To study the effects of cholesterol level in diets on growth, survival, enzyme activity, and disease resistance of larval hybrid lemon fin barb.

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