UNIVERSITI PUTRA MALAYSIA

DIETARY PROTEIN REQUIREMENT OF LEMON FIN BARB HYBRID (Barbonymus gonionotus Bleeker 1849 ♀ × Hypsibarbus wetmorei Smith 1931 ♂) FINGERLINGS

SUHARMILI BINTI ROSLE

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By

SUHARMILI BINTI ROSLE

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

June 2017

DEDICATION

"ACTION IS THE FOUNDATIONAL KEY TO ALL SUCCESS"

This thesis is dedicated to my family members

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

DIETARY PROTEIN REQUIREMENT OF LEMON FIN BARB HYBRID (*Barbonymus gonionotus* Bleeker 1849 ♀ × *Hypsibarbus wetmorei* Smith 1931 ♂) FINGERLINGS

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SUHARMILI BINTI ROSLE

June 2017

Chairman : Mohd Salleh bin Kamarudin, PhD
Faculty : Agriculture

Lemon fin barb hybrid (female *Barbonymus gonionotus* × male *Hypsibarbus wetmorei*) was first produced in 2004 with a novel intention as a poor man's fish. At present, the fish is commercially raised with tilapia or catfish diets since no specific diet has been developed for the hybrid. As protein is the most important and costliest component in an aquafeed, the dietary protein requirements of lemon fin barb hybrid fingerlings were investigated in this study which included the optimal dietary protein and energy requirements, the effectiveness of defatted black soldier fly (*Hermetia illucens*) meal, poultry offal meal and soybean meal as fishmeal replacement and the apparent digestibility coefficients (ADC) of selected feedstuffs. Practical test diets were used in all feeding trials and the effects of these diets on the growth performance, proximate body composition, nutrient and amino acid retentions, and histopathological parameters of the fish were determined. In Experiment 1, five isocaloric (16 kJ g^{-1}) diets containing varying protein levels ranging from 20 to 40% were tested. Six diets with three dietary protein levels (25, 30 and 35%) and two energy levels (16 and 17 kJ g^{-1}) were evaluated in Experiment 2 while the performances of five isonitrogenous (30% protein) and isocaloric (17 kJ g^{-1}) diets with varying dietary level of defatted black soldier fly pre-pupae (BSFP) meal (0, 25, 50, 75 and 100%) as fishmeal replacement were assessed in Experiment 3. In Experiment 4 and 5, similar experimental protocols as in Experiment 3 were adopted in which poultry offal meal (POM) and soybean meal were tested as fishmeal replacement, respectively. Best diets from Experiments 3, 4 and 5 were simultaneously evaluated in Experiment 6 while in Experiment 7, the apparent digestibility coefficient (ADC) of dry matter, nutrients and energy of fishmeal, defatted BSFP, defatted POM, defatted soybean meal, rice bran and corn meal, and their apparent availability coefficent (AAC) of amino acids in the hybrid fish were studied.

The fish growth and body composition in Experiment 1 indicated that the optimal dietary protein requirement of lemon fin barb hybrid fingerlings was 34.6% at 16 kJ g^{-1} gross energy. In the subsequent experiment, the best fish performance was observed at a lower 30% protein and a higher 17 kJ g^{-1} gross energy with a protein-energy ratio of 16.64 mg protein kJ^{-1}. In Experiment 3, it was found that defatted BSFP meal could replace up to 75% fishmeal without affecting fish survival, growth performance, body composition, nutrients and amino acid retentions and histopathological parameters. Similar findings were made in Experiment 4 when poultry offal meal was used as the fishmeal replacement. In Experiment 5, soybean meal was found to could only replace up to 50% fishmeal. The findings of Experiments 3-5 indicated a minimal of 2.5 and 5% fishmeal were required in the hybrid fish diet when animal and plant based protein sources were used as partial fishmeal substitute, respectively. When the best diets from Experiment 3-5 were simultaneously tested, defatted BSFP as partial fishmeal replacement gave the best growth performance followed by defatted POM and defatted soybean meal which suggested that animal proteins were superior to plant proteins for the hybrid. In the digestibility study, the results indicated the lemon fin barb hybrid had a higher digestive capability to utilize animal protein sources especially fishmeal compared to plant based meals. Corn meal was poorly digested by the hybrid fish. In conclusion, the findings of this study provided an important and vital information for the development of a practical specific diet for lemon fin barb hybrid.

Abstrak thesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KEPERLUAN PROTEIN ANAK KERAI LAMPAM
(*Barbonymus gonionotus* Bleeker 1849 ♀ × *Hypsibarbus wetmorei* Smith 1931 ♂)

Oleh

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Kerai lampam (betina *Barbonymus gonionotus* × jantan *Hypsibarbus wetmorei*) telah mula dihasilkan pada tahun 2004 dengan tujuan murni untuk dijadikan sebagai ikan rakyat. Pada masa kini ikan ini dikultur secara komersial menggunakan diet tilapia atau keli disebabkan tiada diet spesifik telah dihasilkan bagi hibrid ini. Oleh kerana protein merupakan komponen penting dan termahal dalam sesuatu makanan ikan, keperluan protein bagi kerai lampam telah disiasat dalam kajian ini termasuk keperluan optimal protein dan tenaga, keberkesanan tepung pupa lalat (*Hermetia illucens*) yang dinyah lemak, tepung perut ayam dan tepung kacang soya sebagai pengganti tepung ikan dan pekali kebolehcernaan nyata (ADC) bagi bahan makanan terpilih. Diet praktikal telah digunakan dalam semua ujian makanan dan kesan diet terhadap prestasi pertumbuhan, komposisi proksimat badan, retensi nutrien dan asid amino, dan parameter histopatologi ikan berkenaan telah ditentukan. Dalam Eksperimen 1, lima diet isokalori (16 kJ g^{-1}) dengan paras protein yang berbeza di antara 20 hingga 40% telah diuji. Enam diet dengan tiga paras protein (25, 30 dan 35%) dan dua paras tenaga (16 dan 17 kJ g^{-1}) telah dinilai dalam Eksperimen 2 sementara lima diet isonitrogenus (30% protein) dan isokalori (17 kJ g^{-1}) dengan berbeza paras tepung pre-pupa lalat *black soldier* (BSFP) yang dinyah lemak (0, 25, 50, 75 dan 100%) sebagai pengganti tepung ikan telah dinilai di Eksperimen 3. Dalam Experimen 4 dan 5, protokol eksperimen dalam Eksperimen 3 telah digunakan di mana tepung perut ayam (POM) dan tepung kacang soya masing-masing diuji sebagai pengganti tepung ikan. Diet terbaik daripada Eksperimen 3, 4 dan 5 telah dinilai secara serentak dalam Eksperimen 6 sementara dalam Eksperimen 7, pekali kebolehcernaan nyata (ADC) bagi bahan kering, nutrien dan tenaga tepung ikan, tepung BSFP, POM dan kacang soya yang dinyah lemak, dedak beras dan tepung jagung, serta pekali keberadaan nyata (AAC) amino asid dalam ikan hibrid ini telah dikaji.

Pertumbuhan dan komposisi badan ikan dalam Ekperimen 1 menunjukkan bahawa keperluan protein yang optima bagi anak kerai lampam adalah 34.6% pada 16 kJ g^{-1} tenaga kasar. Dalam eksperimen seterusnya, prestasi terbaik ikan dilihat pada paras 30% protein dan paras tenaga kasar 17 kJ g^{-1} serta nisbah protein-tenaga 16.64 mg protein kJ^{-1}. Dalam Eksperimen 3, didapati BSFP yang dinyah lemak berupaya menggantikan sehingga 75% tepung ikan tanpa menjejaskan kemandirian, prestasi pertumbuhan, komposisi badan, retensi nutrien dan asid amino serta parameter histopatologi ikan hibrid tersebut. Penemuan yang serupa telah didapati dalam Experimen 4 di mana tepung perut ayam digunakan sebagai pengganti tepung ikan. Dalam Eksperimen 5, tepung kacang soya didapati hanya berupaya menggantikan sehingga 50% tepung ikan. Hasil penemuan Eksperimen 3-5 menunjukkan minimum 2.5 dan 5% tepung ikan adalah diperlukan dalam diet ikan hibrid ini jika sumber protein berasaskan haiwan dan tumbuhan digunakan sebagai pengganti separa tepung ikan, masing-masing. Apabila diet terbaik daripada Eksperimen 3-5 diuji serentak, tepung BSFP yang dinyah lemak sebagai pengganti separa tepung ikan telah memberikan prestasi pertumbuhan terbaik, diikuti oleh POM dan kacang soya yang menunjukkan sumber protein haiwan adalah lebih baik dari sumber protein tumbuhan untuk kerai lampam. Dalam kajian kebolehcernaan, keputusan menunjukkan kerai lampam mempunyai keupayaan kebolehcernaan yang tinggi untuk menggunakan sumber protein haiwan terutamanya tepung ikan berbanding bahan makanan berasaskan tumbuhan. Tepung jagung tidak dicerna dengan baik oleh ikan hibrid ini. Kesimpulannya, hasil kajian ini telah memberikan maklumat penting dan perlu ke arah pembangunan diet praktikal yang khusus untuk kerai lampam.

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I certify that a Thesis Examination Committee has met on 13 June 2017 to conduct the final examination of Suharmili binti Rosle on her thesis entitled "Dietary Protein Requirement of Lemon Fin Barb Hybrid (*Barbonymus gonionotus* Bleeker 1849 ♀ x *Hypsibarbus wetmorei* Smith 1931 ♂) Fingerlings" 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 Doctor of Philosophy.

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LIST OF ABBREVIATIONS

AAC	Apparent availability coefficient
ADC	Apparent digestibility coefficient
ANOVA	Analysis of Variance
BCAA	Branched-chain amino acids
BSFP	Black soldier fly pre-pupae
$Ca(H_2PO_4)H_2O$	Monocalcium phosphate monohydrate
CaCo	Calcium carbonate
Cm	Centimeter
$CoSO_4$	Cobalt(II) sulfate
$CuSO_4.5H_2O$	Copper(II) sulfate pentahydrate
D.O	Dissolved oxygen
DFI	Daily feed intake
E	Energy
EAA	Essential amino acid
FBW	Final body weight
FCR	Food conversation ratio
$FeSO_47H_2O$	Ferrous sulfate heptahydrate
FI (% BW d^{-1})	Feed intake (percentage of body weight per day)
g	Gram
GE	Gross energy
g kg^{-1}	Gram per kilogram
g MJ-^{1}	Gram per megajoule
h	Hours
H_2O_2	Hydrogen peroxide

H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HSI	Hepatosomatic index
IBW	Initial body weight
IU g^{-1}	International unit per gram
Kcal Kg^{-1}	Kilocalorie per kilogram
KCl	Potassium chloride
KI	Potassium iodide
Kg^{-1}	per kilogram
kJ g^{-1}	Kilojoule per gram
L	Liter
M	Molarity
MgOH	Magnesium hydroxide
mg kcal $^{-1}$	Miligram per kilocalorie
mg l $^{-l}$	Milligram per liter
MJ GE kg^{-1}	Megajoule gross energy per kilogram
Mm	Milimeter
mM	Molar mass
ml	Mililiter
ml l^{-1}	Mililiter per liter
$MnSO_4.H_2O$	Manganese sulfate monohydrate
mμ	Milimicron
Na_2HPO_4	Sodium hydrogen phosphate

Na_2SeO_3	Sodium selenite
NaCl	Sodium chloride
NaF	Sodium fluoride
NaOH	Sodium hydroxide
NEAA	Non-essential amino acid
NFE	Nitrogen free extract
nm	Nanometer
P	Protein
PBM	Poultry by-product meals
POM	Poultry offal meal
P:E	Protein energy ratio
PER	Protein efficiency ratio
PUFA	Polyunsaturated fatty acids
RPM	Revolutions per minute
SAS	Statistical Analysis System
SD	Standard deviation
SGR	Survival growth rate
T	Rearing period
USA	United States of America
USD	US Dollar
VSI	Viserosomatic index
WG	Weight gain
$ZnSO_4.7H_2O$	Zinc sulfate heptahydrate
%	Percentage
Ø	Diameter

^{0}C	Degree Celsius
µm	Micrometer
% d^{-1}	Percentage per day
<	Less than
>	More than

CHAPTER 1

GENERAL INTRODUCTION

The role that fisheries play in both the food and nutritional security of many rural and coastal populations has often been underestimated in the past (Lymer *et al.*, 2010). Fisheries industry is partly responsible in providing food security to the growing human population as fish is continuously becoming the required source of animal protein to the community (Ghee-Thean *et al.*, 2016). Globally, fish provide about 3.0 billion people with almost 20% of their intake of animal protein and 4.3 bilion people with about 15% of such protein (Kim, 2015). It is due to the fact that fish contain high nutritional values and provide high quality protein with wide variety of vitamins and minerals including vitamins A and D, phosphorus, magnesium, selenium, and iodine (FAO, 2010).

Fish become important in the diets and livelihoods of many poor people suffering from vitamin and mineral deficiencies (Roos *et al.*, 2007). Fish contain valuable essential fatty acids and amino acids particularly in lysine and its protein is easily digestible (Lymer *et al.*, 2010). Humans require n-3 PUFA in the diet and generally fish oils are rich in n-3 PUFA (Randall *et al.*, 1990). It is apparent that fish make a valuable contribution to the nutritional quality of the diets for human populations of many developing countries in the Asia-Pacific regions (Lymer *et al.*, 2010). In some countries such as Oceania, North America, Europe, Asia, Latin America and the Caribbean, fish is the main protein source with annual consumption of 27.0 kg, 23.6 kg, 20.8 kg, 23.5 kg and 10.0 kg capita^{-1}, respectively (FAO, 2016). FAO (2016) projected apparent fish consumption will increase in 2025 to 21.8 kg (live weight equivalent) capita^{-1}, 8% above the base period level of 20.2 kg capita^{-1}.

The world population is projected to reach 8.1 billion in 2025 and 9.6 billion in 2050. Thus, it is a daunting challenge to ensure adequate food and nutrition securities for this growing population (FAO, 2015). In 2014, the total global capture fisheries production marked a new maximum production at 93.4 million tons compared to 86.9 million tons in 2013 with aquaculture production of fish accounted for 44.1% (FAO, 2015, 2016). It is projected that the total production will reach 196 million tons in 2025 to fulfill the demand (FAO, 2016). Since two decades ago, fisheries has been an important contributing factor in Malaysia, particularly for the rural populations as they provide the source of income, gain of foreign exchange, employment, export potential and provide the main source of protein (Abu Talib & Alias, 1997). Apart from fisheries sector, aquaculture sector also present bright development and expansion when global aquaculture production in 2014 attained 73.8 million tons (FAO, 2016). The global aquaculture industry is not only effectively alleviating stress on natural fish resource, but it creates a source of income, supplies food, generates thousands of employments and revenues, provides healthy protein to human at a reasonable cost and also improves food safety and security (FAO, 2010; Olsen & Hasan, 2012).

Freshwater fishes are reported to be the most threatened group of vertebrates harvested by man (Lundgren *et al.*, 2006; Reid *et al.*, 2013). These researchers concluded that half of the world's species have been lost during the last century due to the construction of dams, major river diversions and canals. The depletion of wild fish stocks has caused more difficulties for hatcheries to find new breeders as many hatcheries often rely on too few breeders to reproduce. This has resulted in lower production, susceptibility to diseases, inbreeding depression, production of small or stunted fish stocks due to no variety of genetic material in fish and poor survival rates in the wild (Chew & Zulkafli, 2012)**.**

In contemporary aquaculture, nutritionally complete feeds that contain all essential nutrients must be fed to fish (Bolorunduru, 2002). Lovell (1991) stated that these nutrients may come from natural aquatic organisms or from prepared diets. Maina *et al.* (2002) stated that the growth and survival of the fish in captivity depend on the nutrients in the feed given since there are very minimal or no natural sources of nutrition can be consumed. The authors suggested that the nutrient composition of feedstuffs, the biological availability of nutrients and energy in each of the ingredients for the species shall be known as they become the potential ingredients in formulating practical diets for fish. Knowledge of the optimum dietary levels of all nutrients is vital for the formulation of a nutritionally balanced low-cost diet for feeding fish (Erfanullah & Jafri, 1998).

Protein is a major component in the fish diet as it promotes growth, health and involved in reproduction and body maintenance. None of the body cells can survive without an adequate supply of protein and protein makes about 20% of the cell mass (Neaves, 2002). Fuller (2004) stated that most herbivorous and omnivorous species require less dietary protein (25-35%) than some carnivorous species (40-50%). As protein constitutes the highest cost in fish feed, it is necessary to determine the best requirement level for survival and growth of fish (Lee *et al.*, 2003). Ali *et al.* (2009) stated that fish consume proteins for energy, however because of expensiveness of proteins, fats and carbohydrates are chosen as source of energy.

Proteins is vital for growth of fish (Lovell, 1991). However, an insufficient protein in the diet causes poor growth (Mohanta *et al.*, 2008). On the other hand, only a portion of protein is used for production of new proteins and the surplus are transformed to energy if excess protein is present in the diet (Halver & Hardy, 2002). Besides, use of protein beyond the required level leads to a higher ammonia production which will affect the water quality, intake of feed and growth performance in fish (McGoogan & Gatlin, 2000). The efficiency of protein consumption for energy is mainly attributed to the way in which the excretion of ammonia from deaminated protein via the gills. (Fuller, 2004). The consumption protein by animals depend on the category of diet, protein digestibility, amino acid content, the dietary energy to ptotein ratio and the quantity of protein provided (Mohanta *et al.*, 2008). Besides, Lim *et al.* (1979) mentioned that sex, size, genotype of animal and ecological conditions influence protein utilization.

Cyprinidae is a family of freshwater fishes that includes true carps or minnows and members of this family are also known as cyprinids (Ur-Rehman *et al.*, 2015). In 2014, the culture of non-fed species increased to 22.7 million tons from 20.5 million in 2012 (7.1 million tons of carps), representing 30.8% of global production of all farmed fish species (FAO, 2014, 2016). The carp production is expected to increase from 15 to 20 million tons in 2030 (The World Bank, 2013; FAO, 2016). In 2015, the production from freshwater aquaculture in Malaysia was 112,145.15 tons compared to 106,731.41 tons in 2014 (DOF, 2015, 2016). The production of carp in 2015 was only 7,379.27 tons that is less than 7% of the total freshwater aquaculture production (DOF, 2016). Rohu, common carp, silver barb and bighead carp dominated the production with a slightly over 90% of the total production.

Nowadays, carps have become popular among fish farmers. Their introduction in many countries have helped to make carps the most widely distributed freshwater fish in the world (McGrouther, 2012). *Hypsibarbus* carps, a relatively new taxon in Cyprinidae, are generally found in South East Asia (Rainboth, 1996). They are considered to be the most closely related to the genera *Barbodes* and *Propuntius* (Ogata *et al.*, 2010). In Thailand, they are found in the main streams of large rivers such as Maeklong, Mekong and Chao Phraya basins (Jantrarotai *et al.*, 2007). Rainboth (1996) reported that there are six *Hypsibarbus* species in Thailand which are *H. lagleri, H. malcolmi, H. salweenensis, H. suvattii*, *H. vernayi* and *H. wetmorei*. Lemon fin barb, *Hypsibarbus wetmorei* is an omnivore (Rainboth, 2012). It also consumes some vegetable matters and occasionally eats aquatic plants in the pond (DOF, 2012). Morphologically, *Hypsibarbus wetmorei* is fairly the same to the silver barb appearance, *Barbonymus gonionotus* except for the of reddish brown upper body with pelvic and anal fins red to reddish orange (Rainboth, 1996) and given a vernacular name of kerai kunyit (Chew & Zulkafli, 2012).

Silver barb, *Barbonymus gonionotus* is an important carp species cultured in Indonesia, Thailand, Vietnam and Malaysia. this fish is an omnivore, feeding mainly on algae and submerged weeds (Mohanta *et al.*, 2009). The fish grows to 700-800 g in a year when cultured in ponds and attains the sexual maturity in 8-10 months for females and 6-8 months for males. The fish is considered marketable when it reaches over 300 g. Females generally grow faster than males (Mohanta *et al.*, 2008).

Considering the potential of these two species as aquaculture candidate, the Department of Fisheries Malaysia through the Perlok Aquaculture Extension Centre have succesfully bred male *H. wetmorei* x female *B.gonionotus* in 2004 (DOF, 2012). This hybrid is not sterile. The F1 broodstock of 200-250 g and more are routinely used for breeding purposes. In ponds, the fry can achieve a 500-600 g body weight within a 6-month culture period. Due to the availability of this hybrid all year long and its high price, its aquaculture had gained interest among fish farmers. The hybrid is commonly fed with tilapia or catfish diet since no specific diet for the fish has been developed.

Lemon fin barb hybrid was introduced with a novel intention as a poor man's fish. Its production in 2015 was 103.44 tons with wholesale value of RM 3.53 million, a drop in production compared to 151.83 tons in 2014 with wholesale value of RM 5.08 million (DOF, 2015, 2016). Despite a drop in the production in 2015, lemon fin barb hybrid has shown an increasing production trend over the last 5 years as the interest among the small-scaled fish farmers has been overwhelming. The main bottleneck in the expansion of this new fish species is the absence of a specific commercial diet for the hybrid. At present, the farmers have been using various commercial feeds for tilapia and catfish and supplemented with plant materials such as napier grass and aquatic plants to raise this fish which may not be optimum or less economical.

No research has been conducted on the dietary protein requirement of lemon fin barb hybrid fingerlings by any researcher. To date, only its optimal dietary lipid requirements and fish oil replacement had been determined (Kamarudin *et al.*, 2015; Ismail *et al.*, 2016). Hence, this study was conducted as an initial step towards the development of a specific practical diet for lemon fin barb hybrid fingerlings. The specific objectives of this study were:

1. To determine the optimal dietary protein requirement of lemon fin barb hybrid fingerlings
2. To determine the optimal protein to energy ratio of lemon fin barb hybrid fingerlings
3. To determine the effectiveness of defatted black soldier fly pre-pupae (*Hermetia illucens*) meal, defatted poultry offal meal and defatted soybean meal as fishmeal replacement in the diet of lemon fin barb hybrid fingerlings
4. To compare the effectiveness of defatted black soldier fly pre-pupae meal, defatted poultry offal meal and defatted soybean meal as fishmeal partial replacement in the diet of lemon fin barb hybrid fingerlings
5. To determine the apparent digestibility coefficients of selected feedstuffs in lemon fin barb hybrid fingerlings

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