UNIVERSITI PUTRA MALAYSIA

DIETARY PROTEIN REQUIREMENT AND FISHMEAL REPLACEMENT IN TINFOIL BARB (Barbonymus schwanenfeldii BLEEKER 1853) FINGERLINGS

NURULJANNAH BINTI MOHD PUAAD

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By

NURULJANNAH BINTI MOHD PUAAD

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Chair : Prof. Mohd Salleh Bin Kamarudin, PhD
Faculty : Agriculture

Tinfoil barb, *Barbonymus schwanenfeldii* (Bleeker, 1854) is an indigenous cyprinid that has a big potential in the global ornamental fish industry and aquaculture. However, its nutrition and nutrient requirements have not been fully studied and understood while specific commercial feed for this species is unavailable. Protein is the most expensive nutrient in a diet and plays an important role in fish growth. In this study, three experiments were conducted to examine the protein requirements of the barb. An 8-week feeding trial was conducted in the Experiment 1 to determine the optimal dietary protein requirement for tinfoil barb (*B. schwanenfeldii*) fingerlings. Six isocaloric (17 kJ g-1 gross energy) diets were formulated to contain 25, 30, 35, 40, 45 and 50 % protein. Fingerlings (0.40 ± 0.03 g) were randomly stocked in 60 L glass aquaria at 25 fish per aquarium. Triplicate groups of fish were fed twice a day until satiation. The results showed that dietary protein level had significant effects on weight gain, specific growth rate, feed conversion ratio and protein efficiency ratio in tinfoil barb. Survival was not affected by the dietary protein level. Weight gain was significantly higher at 50% dietary protein (663.99 ± 24.51 %) compared to those of lower levels. Fish fed with 50% protein also showed significantly higher ($P<0.05$) specific growth rate (3.68 ± 0.30 % d^{-1}) compared to fish fed 25-40 % protein. Feed efficiency improved with the increase in dietary protein level. FCR was less than 1.35 among fingerlings fed 45-50 % of dietary protein whereas fingerlings fed with 20-35 % protein had FCR of 1.7-2.0. In Experiment 2, six test diets were formulated to contain 40, 45 and 50 % protein with 17 and 18 kJ g-1 gross energy to determine if the optimal dietary protein of tinfoil barb could be reduced at a higher dietary energy. Similar rearing and feeding protocols as in Experiment 1 were adopted. The results showed that a higher dietary energy did not have a significant effect on final weight, weight gain, specific growth rate and feed efficiency of tinfoil barb. However, dietary protein had significant effects on fish growth (526.99 ± 30.35 %) and

feed efficiency (1.32 ± 0.15) while its interaction with energy had significant effect on growth. Fish survival was not affected by the dietary protein and energy level, and their interaction. Fish fed 50% protein and 17 kJ g-1 gross energy showed significantly highest ($P<0.05$) specific growth rate (3.34 ± 0.12 % d^{-1}) compared to fish fed with 40% protein and 17-18 kJ g-1 gross. Experiment 3 was conducted to evaluate the performance of fermented soybean meal as fish meal replacement in an effort to reduce feed cost. Five test diets were formulated to replace fish meal at 0, 25, 50, 75 and 100 %. The replacement level had a significant effect on final weight (4.43 ± 0.41 g), weight gain (455.34 ± 18.38 %), specific growth rate (2.79 ± 0.19 – 3.10 ± 0.29 % d^{-1}), DFI (3.12 ± 0.19 – 3.18 ± 0.21 % BW d^{-1}), FCR (1.25 ± 0.15) and PER (1.62 ± 0.18) but not CF, HSI and VSI. The results showed that fermented soybean meal could only replace up to 25% fishmeal in the diet of tinfoil barb. In conclusion, tinfoil barb had a high minimum dietary protein requirement of 45% at 17 kJ g-1 gross energy with fishmeal as a major protein source while fermented soybean meal could only replace up to 25% fishmeal in its diet.

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

KEPERLUAN PROTEIN DAN PENGGANTIAN TEPUNG IKAN DALAM MAKANAN ANAK LAMPAM SUNGAI (*Barbonymus schwanenfeldii* BLEEKER 1853)

Oleh

NURULJANNAH BINTI MOHD PUAAD

Jun 2022

Pengerusi : Prof. Mohd Salleh Bin Kamarudin, PhD
Fakulti : Pertanian

Lampam sungai, *Barbonymus schwanenfeldii* (Bleeker, 1854) ialah sejenis siprinid tempatan yang mempunyai potensi besar dalam industri ikan hiasan dan akuakultur global. Walau bagaimanapun, keperluan pemakanan dan nutriennya belum dikaji dan difahami sepenuhnya manakala makanan komersial khusus untuk spesies ini belum wujud. Protein adalah nutrien yang paling mahal dalam diet dan memainkan peranan penting dalam pertumbuhan ikan. Dalam kajian ini, tiga eksperimen telah dijalankan untuk mengkaji keperluan protein lampam ini. Satu percubaan pemakanan selama 8 minggu telah dijalankan dalam Eksperimen 1 untuk menentukan keperluan protein pemakanan yang optima untuk anak ikan lampam sungai (*B. schwanenfeldii*). Enam diet isokalorik (17 kJ g-1 tenaga kasar) telah dirumuskan untuk mengandungi 25, 30, 35, 40, 45 dan 50 % protein. Anak ikan lampam sungai (0.40 ± 0.03 g) distok secara rawak dalam akuarium kaca 60 L pada kadar 25 ekor setiap akuarium. Anak ikan lampam sungai diberi makan dua kali sehari sehingga kenyang. Keputusan menunjukkan bahawa paras protein diet mempunyai kesan yang ketara ke atas pertambahan berat badan (663.99 ± 24.51 %), kadar pertumbuhan spesifik (3.68 ± 0.30 % d^{-1}), nisbah penukaran makanan dan nisbah kecekapan protein dalam lampam sungai. Kemandirian tidak terjejas oleh paras protein. Pertambahan berat badan adalah ketara lebih tinggi pada diet 50% protein berbanding dengan paras yang lebih rendah. Ikan yang diberi 50% protein juga menunjukkan kadar pertumbuhan spesifik yang lebih tinggi ($P<0.05$) berbanding ikan yang diberi 25-40 % protein. Kecekapan makanan bertambah baik dengan peningkatan paras protein diet. FCR adalah kurang daripada 1.35 dalam kalangan anak ikan yang diberi 45-50 % protein manakala anak ikan yang diberi 20-35 % protein mempunyai FCR 1.7-2.0. Dalam Eksperimen 2, enam diet ujian telah dirumuskan untuk mengandungi 40, 45 dan 50 % protein dengan 17 dan 18 kJ g-1 tenaga kasar untuk menentukan sama ada paras keperluan protein optimum lampam sungai

boleh dikurangkan dengan paras tenaga yang lebih tinggi. Protokol pemeliharaan dan pemberian makanan adalah serupa seperti dalam Eksperimen 1. Keputusan menunjukkan bahawa paras tenaga yang lebih tinggi tidak mempunyai kesan yang ketara ke atas berat akhir, pertambahan berat, kadar pertumbuhan spesifik dan kecekapan makanan lampam sungai. Walau bagaimanapun, paras protein mempunyai kesan yang ketara terhadap pertumbuhan ikan (526.99 ± 30.35 %) dan kecekapan makanan (1.32 ± 0.15) manakala interaksinya dengan tenaga mempunyai kesan yang ketara terhadap pertumbuhan. Kemandirian ikan tidak terjejas oleh paras protein dan tenaga, dan interaksi mereka. Ikan yang diberi makan 50% protein dan 17 kJ g-1 tenaga kasar menunjukkan kadar pertumbuhan spesifik tertinggi (3.34 ± 0.12 % d^{-1}) yang ketara ($P<0.05$) berbanding ikan yang diberi makan 40% protein dan 17-18 kJ g-1 tenaga kasar. Eksperimen 3 telah dijalankan untuk menilai prestasi tepung kacang soya yang difermentasi sebagai pengganti tepung ikan dalam usaha mengurangkan kos makanan. Lima diet ujian telah dirumuskan untuk menggantikan tepung ikan pada 0, 25, 50, 75 dan 100 %. Paras penggantian mempunyai kesan yang ketara ke atas berat akhir (4.43 ± 0.41 g), penambahan berat (455.34 ± 18.38 %), kadar pertumbuhan spesifik (2.79 – 3.10 % d^{-1}), DFI (3.12 – 3.18 % BW d^{-1}), FCR (1.25 ± 0.15) dan PER (1.62 ± 0.18) tetapi bukan CF, HSI dan VSI. Keputusan menunjukkan bahawa tepung kacang soya yang difermentasi hanya boleh menggantikan sehingga 25% tepung ikan dalam diet lampam sungai. Kesimpulannya, lampam sungai mempunyai keperluan protein minimum yang tinggi iaitu 45% pada 17 kJ g^{-1} tenaga kasar dengan tepung ikan sebagai sumber protein utama manakala tepung kacang soya yang difermentasi hanya boleh menggantikan sehingga 25% tepung ikan dalam diet lampam sungai.

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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:

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

Muhammad Fadhil Syukri 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 September 2022

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory 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: Prof. Dr. Mohd Salleh Kamarudin

Signature: _____
Name of Member of Supervisory Committee: Dr. Muhammad Fadhil Syukri Ismail

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
cm	Centimeter
DFI	Daily Feed Intake
DOF	Departments of Aquaculture, Malaysia
DPX	Distyrene, Plasticizer and Xylene mix
H_2SO_4	Sulfuric acid
FAO	Food and Agriculture Organization of the United Nations
FCR	Food Convertion Ratio
g	Gram
GE	Gross Energy
g kg^{-1}	Gram per Kilogram
g mJ^{-1}	Gram per Megajoule
H&E	Haematoxylin and Eosin
HSI	Hepatosomatic Index
kJ g^{-1}	Kilojoule per gram
L	Liter
NaOH	Sodium hydroxide
NFE	Nitrogen Free Extract
NRC	National Research Council, USA
ml	Millimeter
mJ GE kg^{-1}	Megajoule gross energy per kilogram
MUFA	Monounsaturated fatty acids
P:E	Protein: energy

PER	Protein Efficiency Ratio
PUFA	Polyunsaturated fatty acid
SD	Standard deviation
SFA	Saturated fatty acid
SGR	Specific Growth Rate
VSI	Viscerosomatic Index
WG	Weight Gain
%	Percentage
^{o}C	Degree Celcius
Ø	Diameter
µm	Micrometer
% d^{-1}	Percentage per day
<	Less than
>	More than

CHAPTER 1

INTRODUCTION

1.1 Background of study

Cyprinidae is one of the most dominant fish family in global aquaculture and ornamental fish trade. Due to growing demand and pressure on wild resources, the cultivation of carps has expanded in recent decades (Eslamloo et al., 2017)., However, carp remains fourth in Malaysia behind tilapia, clarid catfish and pangasid catfish in the freshwater fish aquaculture production (DOF, 2021). Carp contributed only 7,687 tonnes in 2020 compared to 33,403, 29,012, and 19,648 tonnes by tilapia, clarid catfish and pangasid catfish, respectively.

Tinfoil barb, *Barbonymus schwanenfeldii* (Bleeker, 1854), is an indigenous carp of Malaysia. It is also widely distributed in Brunei, Cambodia, Indonesia, Laos, Malaysia, Singapore, Thailand, and Vietnam and is rapidly becoming commercially important as food fish (Zhang et al., 2017). Known as lampam sungai or tengadak in Malaysia, this fish is also widely reared in South East Asia (Eslamloo et al., 2013). Tinfoil barb spends most of its time between the bottom and the mid-level of the waterways and fish breeds three times per year (Gante et al., 2008). Dewantoro et al. (2018) predicted that tinfoil barb has tremendous economic value and great potential in the aquaculture industry. In the Peninsular Malaysia, its retail price ranged between RM14.00 kg^{-1} to RM19.50 kg^{-1} (DOF, 2021).

Protein is one of the dietary macronutrients and the major component in an aquafeed (Ahmed and Ahmad., 2020). It represents the most expensive component in a diet and plays an important role in fish growth (Meyer & Fracalossi, 2004; Deng et al., 2011). An adequate protein level in the diet is required for the optimal fish growth and any deficiency may lead to reduction or cessation of growth (Wu and Gatlin., 2014; Mohanta et al., 2009). That is why many dietary protein requirement studies have been conducted on fish and crustaceans as the requirement differs with species, size, dietary protein source and environmental conditions (Kim and Lee., 2009). Disproportionate protein contents can affect not only increment of feed costs but also nitrogen loss (Guo et al., 2012). Sagada et al. (2017) suggested that the release of nitrogen from feed into an aquatic system poses environmental concerns and may also impair feeding.

The use of fishmeal in aquafeeds is not sustainable in the future due to its limited supply from the capture fisheries while the global aquaculture industry is rapidly expanding. Researchers have been actively searching for alternative protein sources to reduce its dependency (Huang et al., 2022).

According to Gatlin et al. (2007), plant proteins such as soybean meal, corn gluten meal, rapeseed and wheat protein concentrate are renewable and sustainable protein resources that can potentially reduce the dependency of fishmeal. Among those ingredients, soybean meal is by far the most commonly plant protein used as a fishmeal replacer in aquafeeds and widely used as feed ingredients for many fish species due to balanced amino acid profile and fatty acids (Ma et al., 2019). Despite their huge potential to replace fishmeal, the presence of anti-nutritional factors in these plant meals limit their inclusion levels (Li et al., 2021). Previous studies had demonstrated that fermented soybean meal showed significant improvement in palatability and digestibility compared to native soybean meal (Feng et al., 2022). Besides, fermentation also appears to be a cost-effective approach in aquafeed (Li et al., 2020). There were some previous studies have shown appropriate inclusion of fermented soybean meal improved the performance of fish growth and feed utilization.

1.2 Problem statement

While its wild population is considered as threatened due to intensive fish exploitation (Dewantoro et al., 2018), tinfoil barb is one of slowing growing species despite its great potential in the aquaculture industry. Its highest retail price of tinfoil barb had increased from RM4.00 kg^{-1} in 2014 to RM19.50 kg^{-1} in 2020 (DOF, 2015; DOF, 2021).The aquaculture production of this carp in Malaysia was 159 tonnes in 2012 (DOF, 2013) and rose to a peak of 395 tonnes in 2014 (DOF, 2015). Since then its production has rapid declined to the lowest 10 tonnes in 2016 (DOF, 2017). In 2018, the tinfoil production was only 44 tonnes (DOF, 2019) and further declined to 12 tonnes in 2020 (DOF, 2021). The absence of the specific commercial feed for tinfoil barb is the major bottleneck in its culture expansion. The dietary protein and energy requirements of tinfoil barb fingerlings have not been established and it is crucial to have this information to produce more feasible aquafeeds that can provide its best growth and survival while potentially reduce its production cost.

1.3 Significance of study

Understanding the nutritional needs at every life stage of a fish is essential for developing nutritionally balanced and cost-effective feeds. Meanwhile one of the ways to lower down the feed cost is to reduce the dependency on fishmeal as the main protein source by utilizing sustainable alternative protein sources. This study determined the protein requirements of tinfoil barb fingerlings and evaluated the performance fermented soybean meal. as fishmeal replacement and thus providing vital information towards the development of commercial specific diets for the indigenous carp.

1.4 Objectives

In general, this study was conducted to elucidate the protein nutrition of tinfoil barb fingerlings. Meanwhile the specific objectives were:

i. To determine the dietary protein requirement level of tinfoil barb fingerlings
ii. To establish the optimal protein to energy requirement of tinfoil barb fingerlings
iii. To investigate the effectiveness of fermented soybean meal as fishmeal replacement in the diet of tinfoil barb fingerlings

1.8 Hypothesis

The hypotheses of the study:

Objective 1

Null hypothesis: Increasing dietary protein level did not affect the survival and growth of tinfoil barb fingerlings

Alternative hypothesis: Increasing dietary protein level improved the survival and growth of tinfoil barb fingerlings

Objective 2:

Null hypothesis: Increasing dietary energy level did not lower the dietary protein requirement of tinfoil barb fingerlings

Alternative hypothesis: Increasing dietary energy level lowered the dietary protein requirement of tinfoil barb fingerlings

Objective 3:

Null hypothesis: Fermented soybean meal could fully replace fishmeal in the diet of tinfoil barb fingerlings

Alternative hypothesis: Fermented soybean meal could not or could only partially replace fishmeal in the diet of tinfoil barb fingerlings

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