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UTILIZATION OF DIETARY CARBOHYDRATE IN TINFOIL BARB (Barbonymus schwanenfeldii, Bleeker 1853) FRY

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By

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September 2022

Chairman: **Prof. Mohd Salleh bin Kamarudin, PhD**
Faculty: **Agriculture**

An optimal inclusion of carbohydrates in aquafeeds exerts protein-sparing effect in many farmed fishes and improves their growth. Dietary starch facilitates pellet expansion during feed extrusion and enhances water stability of pellets due to binding capacity. Although freshwater omnivorous fish can better utilize carbohydrate, the efficiency varies with species, and dietary carbohydrate level, source, type and physical form. The present study was conducted to evaluate carbohydrate utilization ability of tinfoil barb fry through five separate feeding trials. Tinfoil barb is an indigenous carp species that has a great potential in aquaculture as a food and ornamental fish. All feeding trials were conducted in 100 L aquaria fitted with top mechanical filters. Fry were stocked at 20 fish per aquarium and fed to satiation twice a day for eight weeks. In the first trial, five isonitrogenous and isocaloric diets with graded levels of corn starch (15, 20, 25, 30 and 35 %) were fed to the fish to determine its optimum starch utilization level. The diets for subsequent feeding trials were prepared with selected native and pregelatinized starch sources, sugars and α-cellulose to substitute corn starch. The fish fed 20% corn starch exhibited highest ($P<0.05$) growth, and feed and protein efficiency. Third order polynomial regression analysis revealed the maximum growth at 19.25% corn starch. At this optimum level, native wheat, taro, tapioca, sago and corn starches had comparable impact on fish growth. However, fish fed taro had lower ($P<0.05$) feed and protein efficiency, and nutrient and energy retention than those fed corn. Dietary taro and wheat groups had a lower ($P<0.05$) midgut α-amylase activity than those fed tapioca. Compared to corn starch, dietary taro shrunk ($P<0.05$) the fish hepatocyte while wheat shrunk its nucleus. Meanwhile, fish fed native and pregelatinized corn, sago and tapioca starches exhibited similar growth, feed efficiency and nutrient retention despite interactive influence of starch source and form ($P<0.05$) on feed digestibility and physical properties. Among the different carbohydrate types, glucose caused poor performance in fish ($P<0.05$) than sucrose, maltose, dextrin and tapioca starch. Growth and midgut α-amylase activity of fish fed 19.25% dietary sucrose was higher ($P<0.05$) than those fed glucose and tapioca starch. Nevertheless, survival of fish fed tapioca was higher ($P<0.05$) after a 14-d challenge test with *Aeromonas hydrophila* than those fed sucrose and dextrin. When the starch content of tinfoil barb diet was substituted with 0, 3, 6, 9 and 12 % α-cellulose, fish growth remained unchanged while α-amylase activity, feed and protein efficiency and protein retention showed increasing trends with rising fiber level. However, based on the overall fish performances, it was concluded that tinfoil barb fry could better utilize native corn, sago and tapioca starches at 19.25% dietary inclusion level. As corn starch is an imported commodity in Malaysia and locally produced sago starch fetched high price, the use of native tapioca starch in tinfoil barb diet was recommended.

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

PENGGUNAAN KARBOHIDRAT DALAM MAKANAN ANAK LAMPAM SUNGAI (*Barbonymus schwanenfeldii*, Bleeker 1853)

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Pemasukan karbohidrat yang optimum dalam makanan akuakultur meningkatkan kualiti fizikal until dan membantu meminimumkan jumlah tepung ikan yang mahal dalam makanan tanpa menjejaskan pertumbuhan ikan. Walaupun ikan omnivor air tawar boleh menggunakan karbohidrat dalam makanan dengan lebih baik, kecekapannya berbeza mengikut spesies serta paras, sumber, jenis dan bentuk fizikal karbohidrat dalam makanan tersebut. Kajian ini dijalankan untuk menilai keupayaan penggunaan karbohidrat juvenil lampam sungai melalui lima ujian pemberian makanan yang berasingan. Lampam sungai adalah spesies kap asal yang mempunyai potensi besar dalam akuakultur sebagai ikan makanan dan hiasan. Semua ujian pemberian makanan telah dijalankan dalam akuarium 100 L yang dilengkapi dengan penapis mekanikal yang dipasang di atas akuarium. Sebanyak 20 ekor anak ikan telah dimasukkan ke dalam setiap akuarium dan diberi makan sehingga kenyang sebanyak dua kali sehari selama lapan minggu. Dalam percubaan pertama, lima diet sama nitrogen dan sama kalori dengan pertambahan aras kanji jagung (15, 20, 25, 30 dan 35 %) telah diberi kepada ikan untuk mengetahui penggunaan kanji optimumnya. Untuk ujian pemakanan yang seterusnya, diet disediakan menggunakan sumber kanji asal dan pra-pengelatinan terpilih, gula dan α-selulosa untuk separa menggantikan kanji jagung. Ikan yang diberi makan 20% kanji jagung menunjukkan pertumbuhan serta kecekapan makanan dan protein tertinggi ($P<0.05$). Analisis regresi polinomial turutan ke tiga menunjukkan pertumbuhan maksimum pada 19.25% kanji jagung. Pada tahap optimum ini, kanji gandum, keladi, ubi kayu, sagu dan jagung asal mempunyai kesan yang serupa terhadap pertumbuhan ikan. Bagaimanapun, ikan yang diberi makan kanji keladi mempunyai kecekapan makanan dan protein ($P<0.05$) serta pengekalan nutrien dan tenaga yang lebih rendah berbanding ikan yang diberi makan kanji jagung. Kumpulan ikan yang diberi makan kanji keladi dan gandum mempunyai aktiviti α-amilase usus tengah yang lebih rendah ($P<0.05$) berbanding ikan yang diberi makan kanji ubi kayu. Berbanding dengan kanji jagung, kanji keladi telah mengecutkan ($P<0.05$) hepatosit ikan manakala kanji gandum telah mengecutkan nukleusnya. Sementara itu, ikan yang diberi makan kanji jagung, sagu dan kanji ubi kayu asli dan pra-pegelatinan mempamerkan pertumbuhan, kecekapan pemakanan dan pengekalan nutrien yang sama walaupun terdapat pengaruh interaktif

sumber dan bentuk kanji (P<0.05) terhadap kebolehcernaan dan sifat fizikal makanan. Antara jenis karbohidrat yang berbeza, glukosa memberikan prestasi buruk (P<0.05) pada ikan berbanding sukrosa, maltosa, dekstrin dan kanji ubi kayu. Pertumbuhan dan aktiviti α-amilase dalam usus tengah ikan yang diberi 19.25% sukrosa adalah lebih tinggi (P<0.05) berbanding yang diberi glukosa dan kanji ubi kayu. Namun begitu, kemandirian ikan yang telah diberi kanji ubi kayu adalah lebih tinggi (P<0.05) selepas diberikan ujian cabaran *Aeromonas hydrophila* selama 14 hari berbanding yang diberi sukrosa dan dekstrin. Apabila kandungan kanji dalam diet lampam sungai digantikan dengan 0, 3, 6, 9 dan 12 % α-selulosa, pertumbuhan ikan kekal tidak berubah manakala aktiviti α-amilase, kecekapan pemakanan dan protein serta pengekalan protein menunjukkan tren peningkatan dengan peningkatan paras serat. Bagaimanapun, berdasarkan prestasi keseluruhan ikan, adalah dirumuskan bahawa anak lampam sungai berupaya menggunakan kanji jagung, sagu dan ubi kayu asal dengan lebih baik pada tahap 19.25% diet. Memandangkan kanji jagung adalah komoditi yang diimport ke Malaysia dan kanji sagu keluaran tempatan berharga tinggi, penggunaan kanji ubi kayu asal dalam diet lampam sungai adalah dicadangkan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

6PF-2-K	6-phophofructo-2-kinase
ACP	Acid phosphatase
ADC	Apparent digestibility coefficient
AKP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BD	Bulk density
BM	Blood meal
BW	Body weight
Cas	Casein
CAT	Catalase
CFU	Colony forming unit
CL	Crude lipid stability
CP	Crude protein stability
Dex	Dextrin
DFI	Daily feed intake
DNA	Deoxyribonucleic acid
DNL	*de novo* lipogenesis
DOA	Department of Agriculture, Malaysia
DOF	Department of Fisheries, Malaysia
DP	Degree of polymerization

IGF-I	Insulin-like growth factor-I
iNSP	Insoluble non-starch polysaccharide
LC_{50}	Lethal Concentration 50
MDA	Malondialdehyde
mRNA	Messenger ribonucleic acid
mtDNA	Mitochondrial deoxyribonucleic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral detergent fiber
NFE	Nitrogen free extract
NRC	National Research Council, USA
NSP	Non-starch polysaccharide
PDI	Pellet durability index
PEP	Phosphoenolpyruvate
PEPCK	Phosphoenolpyruvate carboxykinase
PER	Protein efficiency ratio
PFK-1	6-phosphofructo-l-kinase
PG	Pregelatinized
PK	Pyruvate kinase
PRE	Protein retention efficiency
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SBM	Soybean meal
SFO	Sunflower oil
SGLT	Sodium glucose cotransporter
SGR	Specific growth rate
sNSP	Soluble non-starch polysaccharide

SOD	Superoxide dismutase
SV	Sinking velocity
SW	Seawater
TAOC	Total antioxidative capacity
TGC	Thermal growth coefficient
TSB	Tryptone soya broth
VSI	Viscerosomatic index
WAI	Water absorption index
WG	Weight gain
WS	Water stability
WSI	Water solubility index

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background

Fish is the healthiest source of food in the world with rich nutrient profile and high biological value of protein (Tacon and Metian, 2013) compared to other animal protein sources. Globally, per capita consumption of seafood including finfish, crustaceans and mollusks has increased at 1.3% y^{-1} over the last 59 years with 9 kg in 1961 to 19.8 kg in 2019 (FAO, 2022a, 2021a). In 2019, total world fish production reached 177.8 million tons with the contribution of 85.3 million tons of farmed fishes (Figure 1.1 A) mainly from freshwater aquaculture. Carps, barbels and other cyprinids continue to dominate aquaculture industries (FAO, 2022b, 2020a). Although global fish catch has been stagnating for last two decades (-0.1% y^{-1}) since 2000, a continuous growth in cultured fish production (5.2% y^{-1}) fuels the expanding seafood demand. With the rapid growth rate, farmed fish production is predicted to surpass total fish catch in near future and share 60% of global fish supply by 2030 through blue revolution (Ahmed and Thompson, 2019; Cai and Zhou, 2019; FAO, 2018; Garlock et al., 2020). Meanwhile, Asian aquaculture has already overtaken its capture fishery by 2008 and produced 75.4 million tons of fish in 2019 (Figure 1.1 B), which is 1.5 times of total fish catch in Asia (FAO, 2022b). Despite overall trends, a wide variation exists in regional aquaculture productions and seafood supply due to differences in resource distribution, farming systems, environmental regulations, consumer perceptions and food sovereignty, species choice and diversity (Carrassón et al., 2021; Garlock et al., 2020; Gephart et al., 2021; Ghamkhar et al., 2021; Henriksson et al., 2021; Kobayashi et al., 2015; Metian et al., 2020). For example, though Malaysia represents Asia, its cultured fish production has fallen at 5.5% y^{-1} for a decade since 2010, whereas the annual production has grown at 4.4% in the world and Asia (Figure 1.1 C). On the other hand, an average Malaysian has consumed 56.8 kg of fish in 2018, which is more than double of the world and Asia averages (FAO, 2022b, 2022a). Due to this high consumption, Malaysia annually spends around USD 1.14 billion to satisfy the domestic demand through seafood imports (FAO, 2022a, 2021b). Hence, the country urgently needs to strengthen food fish culture at least to share one-half of its total fish production to sustain the seafood security (Kamarudin, 2015) and to save the deficit in foreign exchange.

Meanwhile, Malaysia continuously endeavors to improve the aquaculture as a promising means for nutrition security and economic development with the fullest institutional supports (Othman et al., 2017; Witus and Vun, 2016). Accordingly, even with the Covid-19 restrictions, the sector was able to produce 0.2 million tons of fish in 2020 as in the last five years, whereas the fish catch dropped by 5% compared to the previous year (DOF, 2021; FAO, 2022b; Waiho et al., 2020). However, expedited aquaculture development requires scientifically proven innovation in fish breeding, rearing condition, disease prevention, biosecurity measures, pollution control, species diversification, and nutrition and feeding (Cooney et al., 2021; Fiorella et al., 2021; Metian et al., 2020; Oyarzún et al., 2020; Shen et al., 2021b; Sicuro, 2021; Tung et al., 2021). More importantly, the insight of blue revolution inculcates mandatory use of nutritionally balanced species-specific aquafeeds for the expansion of aquaculture production (Parish et al., 2020; Tacon, 2020). Currently, Malaysian food fish aquaculture harvests are composed of 29 freshwater fish species and 32 marine species (DOF, 2021) but the productivity of certain indigenous farmed fishes is still low due to lack of appropriate feed to support their maximum growth performance.

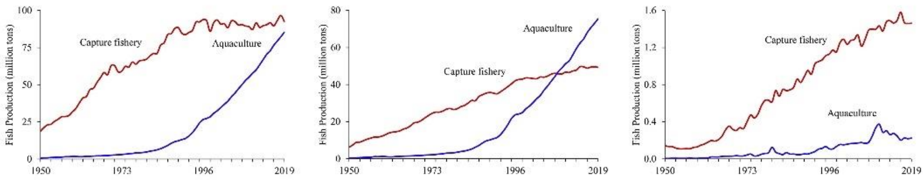

Figure 1.1: Total fish production for last 70 years in (A) the world (B) Asia and (C) Malaysia through capture fisheries and aquaculture (FAO, 2022b)

Tinfoil barb (*Barbonymus schwanenfeldii*) is one of the freshwater omnivorous carps raised in commercial and subsistence food fish farming in Southeast Asia and in the global aquarium fish trades (Alderton, 2019; Jaffar et al., 2019; Maceda-Veiga et al., 2013; Mohsin and Ang, 1979). Due to its pleasing taste, it fetches to as high as RM 150 kg^{-1} particularly in Sarawak (DOF, 2019; Isa et al., 2012). However, its farmed production was only 24.6 tons in 2020, which was 16 times lower than the peak in 2014 and shows a decreasing trend since then primarily due to absence of commercial specific feed and subsequent slow growth (DOF, 2021; Huwoyon and Kusmini, 2010; Mellisa et al., 2021; Suharmili et al., 2015). On the other hand, a steady supply of quality seeds from the government and private hatcheries is ensured through the well-established breeding techniques (Bailey and Cole, 1999; DOF, 2021; Epasinghe et al., 2016; Harmin et al., 1996; Kusmini et al., 2020). However, most small-scale farmers are less motivated on fish farming due to surge of ingredient and feed prices and threats of transboundary fish diseases (Abdullah et al., 2018; Kamarudin, 2015; Othman et al., 2017).

Most studies in the recent past have focused on the formulation of economically viable aquafeeds with cost-effective ingredients to maximize farm profits by minimizing feed cost that usually accounts 65–81 % of the total production cost (Bruni et al., 2021; Cooney et al., 2021; Coutinho et al., 2018; Hasan and Shipton, 2021; Mitra, 2021). Use of plant-based protein sources like soybean meal, algal meal, *Moringa oleifera* seed, leaf meals and others has paved the way to reduce the reliance on expensive fishmeal in aquafeeds (Abdel-Latif et al., 2022; Kumar et al., 2020; Perez-Velazquez et al., 2019, 2018; Teves and Ragaza, 2016). Likewise, the incorporation of digestible carbohydrates enables preparation of environmentally sound least-cost aquafeeds with added advantage on diet quality and fish growth (Kamalam et al., 2017; Maas et al., 2020; Stone, 2003). Although fish are independent on dietary carbohydrates for caloric needs (NRC, 2011), optimal feeding exerts protein-sparing effect through control of hepatic glucose metabolic pathways and improves fish growth, feed efficiency, protein retention and antioxidant capacity (M. Ren et al., 2021; Q. Zhang et al., 2021). Among the freshwater farmed fish species, herbivores and omnivores can efficiently utilize carbohydrates compared to carnivores (Ali and Al-Asgah, 2001; Anand et al., 2018; Tian et al., 2020). Generally, fish are more likely to utilize digestible carbohydrates with complex molecular structure due to enhanced digestive enzyme activities and upregulation of glycolytic gene expressions (M. Li et al., 2021; Ren et al., 2015; W. Xu et al., 2020). Exceptionally, few carnivores like Chinese long snout catfish *Leiocassis longirostris* and rainbow trout *Oncorhynchus mykiss* are able to perform well on dietary sugars (Hung and Storebakken, 1994; Tan et al., 2006). Since the enzymatic hydrolysis of native starches is influenced by the structure and composition of starch granules, fibrous contents and interaction between components (Corgneau et al., 2019; Gao et al., 2020; Honorato et al., 2016; Qi and Tester, 2016; Shi et al., 2017; Svihus et al., 2005), carbohydrate usability by the fish also varies with their botanical origins (Asemani et al., 2019; S. D. Ishak et al., 2021; Jiang et al., 2020; S. Li et al., 2019c). In general, pregelatinization techniques improve utilization of native starches by farmed fish species and enhance physical properties of feed pellets (Romano and Kumar, 2019). The effectiveness of starch physical states on fish performance and feed quality however relies on fish species (Asemani et al., 2019; L. Zhang et al., 2020a), dietary ingredients and their characteristics (Hamid et al., 2011) and feed processing techniques (Kraugerud et al., 2011; Riaz and Rokey, 2012; Welker et al., 2018). Therefore, an efficient aquafeed formulation strategy necessarily needs to encompass all variables to reap maximum benefits from dietary carbohydrates both qualitatively and quantitatively.

1.2 Problem statement

The main drawback in formulating a cost-effective diet for tinfoil barb has been lack of scientific studies available on the requirement of major nutrients. Past studies have dealt with energy:protein ratio in the practical diet (Dewantoro et al., 2018), feeding of agricultural by-products (Mansour et al., 2017b; Suryaningrum et al., 2021), vitamin C in the larval diet (Mellisa et al., 2021) and the effects of β-glucan on gut health (Jung-Schroers et al., 2019). In terms of nutrient requirements of this species, Puaad et al. (2018) have estimated its optimal dietary protein level using semi-purified feeds. However, no information is available on dietary requirements of other major nutrients for this species. At this juncture, it is impossible to formulate a nutritionally balanced diet to support rapid growth and development of its farmed production. Further research is therefore of the utmost importance particularly to estimate optimal dietary inclusion levels of lipid and carbohydrates.

In the absence of cost-effective artificial diet for tinfoil barb fry, studies on carbohydrate nutrition can be a stride to advance its specific feed formulation. As a potential omnivorous carp for aquaculture, this species has a high probability to effectively utilize dietary carbohydrates and yield maximum growth performances. Formulation of carbohydrate-free diet on the other hand would not only exhaust expensive dietary protein for energy but also increase nitrogenous waste excretion and reduce pellet quality. This would ultimately affect feed cost and fish production.

1.3 Objectives

In the absence of nutritional information with strong scientific justification, this study was conducted to investigate utilization of dietary carbohydrate in tinfoil barb fry with the following specific objectives;

1. to determine the optimal digestible carbohydrate utilization level in tinfoil barb fry
2. to determine the appropriate starch sources in the practical diet of tinfoil barb fry
3. to determine the suitable physical form of starch in the practical diet of tinfoil barb fry
4. to determine the appropriate types of carbohydrate in the practical diet of tinfoil barb fry
5. to determine the tolerable fiber level in the practical diet of tinfoil barb fry

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