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DIETARY CARBOHYDRATE UTILIZATION BY THE MALAYSIAN MAHSEER, Tor tambroides (BLEEKER, 1854)

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By

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DIETARY CARBOHYDRATE UTILIZATION BY THE MALAYSIAN MAHSEER, *Tor tambroides* (BLEEKER, 1854)

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Chair: Prof. Mohd Salleh bin Kamarudin, PhD
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This study was carried out to investigate the utilization of dietary carbohydrate by the Malaysian mahseer, *Tor tambroides* (Bleeker, 1854). Wild mahseer fingerlings were obtained from fish suppliers in Pahang and Kelantan. Different batches of fish were used in different feeding trials and they were acclimatized for three weeks before the start of these trials. The effects of different experimental diets on the growth performance, feed utilization efficiency, body nutrient composition, nutrient retention, liver and intestine morphology, and glucose-6-phosphate dehydrogenase (G6PD) gene expression were measured. This study also attempted to evaluate the physical properties of extruded diets using selected starch sources for suitability in mahseer feeding.

In the first feeding trial, fish were fed four experimental diets containing four carbohydrate levels (15%, 20%, 25% and 30%) using food grade corn starch as the carbohydrate source for 10 weeks. The best growth performance was observed in fish fed 20-25% dietary carbohydrate. Using a second-order polynomial regression analysis on the fish growth, the optimal dietary carbohydrate requirement of Malaysian mahseer was determined at 23.44%. Subsequent feeding trials were then based on this optimum level.

In the second study, three locally grown starch sources, sago (*Metroxylon sagu*), cassava (*Manihot esculenta*) and taro (*Colocasia esculenta*) were tested as replacement for imported corn starch in the production of extruded feed for mahseer. Results showed that sago starch gave good expansion ratio and floatability and thus suitable for the production of floating mahseer feed. These feeds were then fed to mahseer juveniles for 10 weeks to evaluate the suitability of these starches as dietary carbohydrate and energy source for the Malaysian mahseer. Results showed that fish fed with corn starch and taro starch performed significantly better (P<0.05) than those fed sago and cassava starch. However, fish fed corn starch had the highest body lipid, lipid retention, and intraperitoneal fat indicating high conversion of

carbohydrate to lipid compared to taro starch. Therefore, taro starch seemed to be the best candidate as a full or partial replacement of corn starch for the production of extruded feed for this species.

The third feeding trial was conducted for 12 weeks to determine the effects of three forms of carbohydrates (starch, disaccharide sucrose and monosaccharide glucose) on the growth performance, feed utilization efficiency, body composition, nutrient retention, and liver and intestine morphology of mahseer. Best performance was observed in fish fed starch which suggested that mahseer benefited from higher complexity of carbohydrates compared to simple carbohydrates.

The final feeding trial was conducted for 10 weeks to evaluate the effect of dietary carbohydrate level and form on the regulation of G6PD gene in mahseer. Fish were fed experimental diets prepared from the first and third feeding trials. Using real time PCR assays, the mRNA expression of G6PD gene in fish liver was estimated. Results showed that G6PD gene expression was significantly elevated ($P<0.05$) in the liver of fish fed 20% carbohydrate and maximum G6PD expression was achieved at 22.2% carbohydrate level which was very close to the optimal dietary carbohydrate requirement obtained in the first experiment. Among fish fed different carbohydrate forms, the highest G6PD gene expression ($P<0.05$) was observed in fish fed glucose. Dietary carbohydrate level and form influenced blood glucose level in mahseer which directly regulated the mRNA expression of G6PD gene during carbohydrate metabolism.

A 20-25% dietary carbohydrate inclusion was recommended for the Malaysian mahseer. A higher or lower inclusion would affect the fish growth. This fish preferred dietary starch with corn and taro starches giving a good growth. High G6PD gene expression indicated increased carbohydrate metabolism and blood glucose level were due to the increasing dietary carbohydrate level. In conclusion, this present study strongly indicated that the Malaysian mahseer has a moderate ability in utilizing dietary carbohydrate inclusion level with a preference for soluble polysaccharides and a limited ability in utilizing starch sources.

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

PENGGUNAAN KARBOHIDRAT OLEH KELAH MERAH, *Tor tambroides* (BLEEKER, 1854)

Oleh

SAIRATUL DAHLIANIS BINTI ISHAK

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Penyelidikan ini dijalankan bagi mengkaji penggunaan karbohidrat dalam diet kelah merah (*Tor tambroides*, Bleeker). Anak ikan kelah liar telah diperoleh daripada pembekal ikan dari Pahang and Kelantan. Kumpulan ikan berbeza telah digunakan dalam eksperimen pemakanan yang berlainan dan kesemuanya diaklimatasi selama tiga minggu sebelum kajian pemakanan dimulakan. Kesan diet ujian berbeza terhadap prestasi pertumbuhan, kecekapan penggunaan makanan, komposisi nutrien badan, pengekalan nutrien, morfologi hati dan usus serta ekspresi gen glukosa-6-fosfat dehidrogenase (G6PD) telah diukur. Kajian ini juga telah menilai sifat fizikal diet yang diekstrusi menggunakan sumber kanji terpilih untuk kebolehsuaian dalam pemakanan ikan kelah.

Dalam kajian pemakanan pertama, kelah diberi makan empat diet ujian yang mengandungi empat kadar karbohidrat (15%, 20%, 25% dan 30%) menggunakan kanji jagung selama 10 minggu. Prestasi pertumbuhan terbaik diperhatikan pada ikan yang diberi 20-25% karbohidrat. Melalui analisis regresi polinomial order kedua, keperluan karbohidrat dietari yang optimum ditentukan pada 23.44%. Eksperimen berikutnya adalah berdasarkan paras optimum ini.

Dalam kajian kedua, tiga sumber kanji tempatan, sagu (*Metroxylon sago*), ubi kayu (*Manihot esculenta*) dan keladi (*Colocasia esculenta*) telah diuji untuk menggantikan kanji jagung yang diimport dalam pengeluaran makanan terapung untuk kelah. Keputusan menunjukkan bahawa kanji sagu memberikan nisbah pengembangan dan keapungan yang baik dan sesuai untuk penghasilan makanan kelah terapung. Kesemua diet ini kemudian diberikan kepada kelah selama 10 minggu untuk menilai kesesuaian kanji ujian sebagai sumber karbohidrat dan tenaga untuk kelah. Keputusan menunjukkan bahawa prestasi ikan yang diberi makan dengan kanji jagung dan keladi ketara lebih baik ($P<0.05$) daripada ikan yang diberi kanji sagu dan ubi kayu. Walau bagaimanapun, ikan yang diberi kanji jagung mempunyai lipid badan, pengekalan lipid, dan lemak intraperitoneal yang tertinggi menunjukkan

penukaran karbohidrat kepada lipid yang tinggi berbanding dengan kanji keladi. Kanji keladi kelihatan sebagai calon terbaik untuk penggantian penuh/separa kanji jagung dalam penghasilan makanan terapung untuk spesies ini .

Eksperimen pemakanan ketiga dijalankan selama 12 minggu untuk menentukan kesan tiga bentuk karbohidrat (kanji, disakarida sukrosa dan monosakarida glukosa) terhadap prestasi pertumbuhan, kecekapan pertukaran makanan, komposisi badan, pengekalan nutrien, serta morfologi hati dan usus kelah. Prestasi terbaik diperhatikan pada ikan diberi kanji yang menunjukkan bahawa kelah mendapat manfaat daripada karbohidrat lebih kompleks berbanding karbohidrat mudah.

Eksperimen pemakanan terakhir dijalankan selama 10 minggu untuk menilai kesan perbezaan paras dan bentuk karbohidrat terhadap regulasi gen G6PD dalam kelah. Ikan diberi makan diet ujian yang disediakan dari eksperimen pemakanan pertama dan ketiga. Menggunakan ujian PCR masa nyata, ekspresi mRNA gen G6PD dalam hati ikan dianggarkan. Keputusan menunjukkan bahawa ekspresi gen G6PD mengalami peningkatan signifikan (P<0.05) dalam hati ikan yang diberi makan karbohidrat 20% dan ekspresi G6PD maksimum dicapai pada paras karbohidrat 22.2% yang menghampiri paras keperluan karbohidrat dietari optimum yang diperolehi dalam eksperimen pertama. Di antara ikan yang diberi makan bentuk karbohidrat yang berbeza, ekspresi gen G6PD tertinggi (P<0.05) diperhatikan dalam ikan yang diberi makan glukosa. Paras dan bentuk karbohidrat mempengaruhi paras glukosa dalam darah ikan kelah yang secara langsung mengawal ekspresi mRNA gen G6PD semasa metabolisme karbohidrat.

Paras karbohidrat dalam makanan yang disyorkan untuk kelah adalah 20-25%. Paras yang lebih tinggi atau rendah akan mengganggu pertumbuhan ikan. Kelah memilih kanji yang mana kanji jagung dan keladi memberikan pertumbuhan yang baik. Ekspresi gen G6PD yang tinggi menunjukkan peningkatan metabolisme karbohidrat dan paras glukosa darah disebabkan kenaikan paras karbohidrat dietari. Sebagai kesimpulan, kajian ini menunjukkan bahawa kelah merah mempunyai keupayaan sederhana dalam menggunakan paras karbohidrat dalam makanan dan lebih cenderung memilih polisakarida larut dengan keupayaan penggunaan sumber karbohidrat yang terhad.

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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:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BD	Bulk Density
DEPC	Di-ethyl Pyro-carbonate
DNA	Deoxy-ribonucleic Acid
DOA	Department of Agriculture, Malaysia
DOF	Department of Fisheries, Malaysia
DP	Degree of Polymerization
DPX	Distyrene, Plasticizer and Xylene mix
ER	Expansion Ratio
F	Floatability
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed Conversion Rate
G6PD	Glucose-6-Phosphate Dehydrogenase
H&E	Haematoxylin and Eosin
HPLC	High Performance Liquid Chromatography
HSI	Hepato-somatic Index
HUFA	Highly Unsaturated Fatty Acids
IPF	Intra-peritoneal Fat
LG	Length Gain
mRNA	Messenger Ribo-nucleic Acid
MSE	Mean Standard Error
mtDNA	Mitochondrial DNA
NADPH	Nicotinamide Adenine Dinucleotide Phosphate reduced
NFE	Nitrogen-Free Extract
NRC	National Research Council, USA
PCR	Polymerase Chain Reaction
PDI	Pellet Durability Index
PER	Protein Efficiency Ratio
RAPD	Random Amplification of Polymorphic DNA
RNA	Ribo-nucleic Acid
RT-PCR	Reverse Transcriptase- Polymerase Chain Reaction
rRNA	ribosomal RNA
SEM	Scanning Electron Microscope
SGR	Specific Growth Rate
SV	Sinking Velocity
TD	True Density
VSI	Viscero-somatic Index
WG	Weight Gain
WS	Water Stability

CHAPTER 1

GENERAL INTRODUCTION

The Malaysian mahseer, *Tor tambroides* (Bleeker, 1854), is a freshwater riverine carp species that is generally distributed throughout Southeast Asian countries (Kottelat, 2000, Rainboth, 1996). This species has long been valued as a game fish for anglers, an ornamental fish for fish hobbyists and also an expensive delicacy served in restaurants. As its fisheries had drastically declined, interest to commercially culture mahseer has intensified (Chowdhury *et al.*, 2016, Esa *et al.*, 2011, Ingram *et al.*, 2006, Ismail *et al.*, 2011, Ismail *et al.*, 2012, Ramezani-Fard and Kamarudin, 2012b, Rashid *et al.*, 2015, Siraj *et al.*, 2007a). Mahseer aquaculture production of Malaysia in 2015 was 24.7 tonnes with the wholesale value of RM2.35 million (DOF, 2016). Meanwhile the retail price of live mahseer at the seafood restaurants in Malaysia and Singapore can reach as high as USD 80-200/kg (Kamarudin *et al.*, 2014).

A good practical, formulated diet must contain satisfactory energy, essential amino acids, lipid, vitamins, minerals and other nutrients to meet the nutritional requirements of the cultured species for a maximum growth with the least cost (Hardy and Barrows, 2002). This means that aquafeed manufacturers must balance the nutritional requirements of the cultured species with the availability and cost of the ingredients. Protein and essential amino acids are important components to ensure growth and the expensive protein ingredients make up the bulk of the feed. Meanwhile feed cost is the highest operational cost in fish farming (Gatlin *et al.*, 2007). De Silva and Anderson (1995) pointed out that extensive research to determine the quantitative essential amino acid requirement for each species is not important because estimates of the requirement is sufficient by using crude protein content. This notion encourages the production of commercial protein-rich diets that can be fed to a broad range of species without regards of their nutritional needs. However, excess protein or amino acids in protein-rich diets may be lost as energy or as waste which contributes to the nutrient loading in environment (Trushenski *et al.*, 2006). Hardy and Barrows (2002) stressed the importance of specific feed tailored for each species with the need to add *n-3* and *n-6* highly unsaturated fatty acids (HUFA) in the feed of marine cultured species in comparison to freshwater species. This is because freshwater species is capable in synthesizing *n-3* and *n-6* HUFA *de novo* unlike their marine counterparts (Sargent *et al.*, 2002, Tocher *et al.*, 2001, Tocher, 2003). As fish have no specific dietary carbohydrate requirement, some form of digestible carbohydrate should be included in the diet as it may serve as precursors for dispensable amino acids and nucleic acids (NRC, 1993). Although carbohydrate is a cheap source of energy, the maximum inclusion level of this component should be determined based on species tolerance (De Silva and Anderson, 1995, NRC, 1993, NRC, 2011). By formulating diets specific to its targeted cultured species, unwanted expenditure on unnecessary expensive ingredients can be avoided (Hardy and Barrows, 2002, Trushenski *et al.*, 2006).

Not many studies have been conducted on the dietary nutritional requirements of mahseers including the Malaysian mahseer. In the last decade, only dietary protein and lipid requirements of juvenile Malaysian mahseer have been determined which are 40% and 5%, respectively (Misieng *et al.*, 2011, Ng *et al.*, 2008, Ng and Andin, 2011, Ramezani-Fard *et al.*, 2012b). This species is also able to utilize poultry offal meal and palm oil as fishmeal and fish oil substitutes, respectively (Ismail *et al.*, 2012, Ramezani-Fard and Kamarudin, 2012b, Ramezani-Fard *et al.*, 2012a). However, the utilization of dietary carbohydrate in this species has not been reported.

It is advantageous to utilize cheap sources of digestible energy such as carbohydrates under practical culture conditions (Kaushik, 1995, Mohanta *et al.*, 2009). Majority of the studies are concentrated on fishmeal and fish oil replacements with focus on the use of grains, oilseeds and legumes as they are inexpensive and contain significant protein and lipid contents. However, plant-based protein replacements also contain high amount of carbohydrates and lack of emphasis on the carbohydrate aspect of the replacement ingredients could lead to poor feed utilization by the fish (Stone *et al.*, 2003). Naturally, fish has the ability to utilize certain types of carbohydrates depending on its species, natural habitats, feeding habits, size and age (Shiau, 1997). These metabolic differences may be due to variation in enzyme activity, hormonal secretion and/or digestion capability between species (Wilson, 1994). As an omnivorous fish, Malaysian mahseer should have a better digestion for carbohydrate components.

Corn starch has been used as an aquafeed binder to stabilize pellet from disintegration in water and prevent nutrient leaching (NRC, 1993, Paolucci *et al.*, 2012). It is also an important ingredient for the production of extruded aquafeeds. Although carbohydrate is considered a cheap form of energy compared to fishmeal or fish oil, the price for corn starch and meal has been increasing in recent years because of increased demand on lignocellulosic biomass from corn stover for biofuel (Baker and Zahniser, 2006, Cate, 2016, Loqué *et al.*, 2015). Starch from sago (*Metroxylon sagu*), cassava (*Manihot esculenta*) and taro (*Colocasia esculenta*) can be good candidates as potential substitutes for corn starch as these plants have socio-economic importance in Malaysia and many tropical developing countries (Awg-Adeni *et al.*, 2010, Lebot *et al.*, 2004, Versino and García, 2014).

Excess carbohydrates consumed by fish are often stored in adipose and liver tissues as lipid via pentose phosphate pathway (Kamalam *et al.*, 2016). Glucose-6-phosphate dehydrogenase (G6PD) plays a major role as cytoplasmic enzyme in the pentose phosphate pathway. In liver, G6PD and 6-phosphogluconate dehydrogenase (6PGD) work mutually with malic enzyme in producing NADPH used for lipid biosynthesis, ribonucleic acids biosynthesis and protection against oxidative stress (Dabrowski and Guderley, 2002, NRC, 2011, Salati and Amir-Ahmady, 2001). This gene has been studied for metabolism changes in teleosts (Chen *et al.*, 2013, Deane and Woo, 2005b, Kamalaveni *et al.*, 2001, Qiang *et al.*, 2014b, Zheng *et al.*, 2013).

The objectives of this study were:

(i) To determine the optimal dietary carbohydrate inclusion level for Malaysian mahseer;
(ii) To evaluate the performances of alternative starch sources (taro, cassava, sago) in the practical diet of Malaysian mahseer;
(iii) To assess the performance of Malaysian mahseer fingerling in utilizing selected carbohydrate forms (monosaccharide, disaccharide, soluble polysaccharide);
(iv) To elucidate the G6PD gene regulation in liver of Malaysian mahseer in regards to carbohydrate utilization.

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