CARBOHYDRATE UTILIZATION IN THE JUVENILE HYBRID GROUPER *(Epinephelus fuscoguttatus* ♀× *Epinephelus lanceolatus* ♂*)*

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ABSTRACT

Carbohydrate is one of the cheapest non-protein dietary components in fish diet that helps to metabolize other components and also improves the physical properties of pelleted feed. TGGG *(Epinephelus fuscoguttatus, ♀)* X *(Epinephelus lanceolatus, ♂)*, is a carnivorous fish that naturally has high requirement for the expensive dietary protein in the diet. Finding cheaper alternative energy sources to reduce the amount of protein in the diet is an important investigation in the effort to develop cost-effective diets for the sustainability of grouper farming. Considerable research on carbohydrate utilization in cultured fish has been conducted with inconsistent findings and mostly species-specific. In addition, little is known about the utilization of carbohydrate in TGGG. The present study was carried out to investigate the effects of different carbohydrate sources on fish performance and also to study the protein sparing effects of sago as a source of carbohydrate. In trial 1, four isoproteic (50% crude protein) and isolipidic (10% crude lipid) diets were formulated containing four different carbohydrate sources (tapioca starch, corn starch, sago starch and dextrin) at the same inclusion level (20%) and fed to triplicate groups of TGGG (13.78 ± 0.08 g mean initial weight). The fish were cultured in a flow-through seawater system and hand-fed twice a day with experimental diets until satiation for 67 days. At the end of feeding trial, the body weight gained (BWG), specific growth rate (SGR), and feed conversion ratio (FCR) of starch origins including Diets sago (798.01 %, 3.28 %/day and 1.19, respectively), Corn (782.09 %, 3.25 %/day and 1.17, respectively), and Tapioca (780.90%, 3.24 %/day and 1.15, respectively) were not affected and performed equally well. However, significantly lower BWG and SGR were observed in TGGG fed Diet Dextrin (707.66%, 3.12 %/day) and the finding was correlated with feed intake (FI) as significant reduction of feed intake was observed in Diet Dextrin. The survival of TGGG was 100% in all treatments with no significant difference (*P*>0.05) was detected. In contrast to growth performances, the hepatosomatic index (HSI) and viscerasomatic index (VSI) were significantly higher in Diet Dextrin than starch-based Diets. The whole-body moisture, ash and lipid were affected by dietary treatments. However, protein was not dependent on dietary carbohydrate sources. The glucose and total protein (*P*< 0.05) were significantly affected by dietary carbohydrate sources and no significant differences in cholestrol and triglyceride contents among treatments. As a conclusion, all the tested starches can be successfully used as carbohydrate sources in the diet for TGGG. In Trial 2, five experimental diets were formulated to be isolipidic (10% CL) and sago starch was used as a sole carbohydrate source. Two levels of protein (40 and 45% crude protein) and two levels of carbohydrate (25 and 30% carbohydrate) (coded as 45P 25C, 45P 30C, 40P 25C and 40P 30C, respectively) and Diet with 50% protein and 20% starch (50P20C) was formulated as a control diet. These diets were hand-fed to triplicate groups of TGGG (12.12 ± 0.08 g mean initial weight) for 57 days. The BWG (576.62-618.91%), FCR (1.48-1.59), survival (93.33-98.33%), and body indices except viscerasomatic index were independent of different dietary treatments. Meanwhile, proximate compositions of whole-body fish, liver and muscle were influenced by the different protein and carbohydrate levels. Apparent Digestibility Coefficient including dry matter, protein and lipid were also significantly influenced by the tested diets. The findings suggest that Sago starch has indicated some protein sparing where increasing carbohydrate level up to 30% and reducing protein to 40% was tolerated by TGGG. The present study has provided valuable knowledge to the aquaculture feed industry on different source of carbohydrates that can be used in the diets without deteriorating the fish performance and its potential in reducing the use of expensive protein in the diets.

ABSTRAK

PENGUNAAN KARBOHIDRAT DALAM KERAPU HIBRID (Epinephelus fuscoguttaus x Epinephelus lanceolatus)

Karbohidrat adalah salah satu komponen pemakanan bukan protein yang paling murah dalam diet ikan yang dapat membantu metabolisma komponen lain dan juga memperbaiki sifat fizikal makanan pelet. TGGG (Epinephelus fuscoguttatus, ♀) X (Epinephelus lanceolatus, ♂), adalah ikan karnivor yang secara semulajadi mempunyai keperluan tinggi untuk protein yang mahal di dalam diet. Pencarian sumber tenaga alternatif yang lebih murah untuk mengurangkan jumlah protein di dalam diet adalah satu penyiasatan penting dalam usaha untuk membangunkan diet berkos-efektif bagi kemapanan penternakan kerapu. Sejumlah penyelidikan mengenai penggunaan karbohidrat dalam ikan yang diternak telah dijalankan dengan penemuan yang tidak konsisten dan kebanyakan adalah bersifat spesies-spesifik. Tambahan pula, hanya sedikit yang diketahui mengenai penggunaan karbohidrat pada TGGG. Kajian ini telah dijalankan untuk mengkaji kesan sumber karbohidrat yang berbeza pada prestasi ikan dan juga untuk belajar kesan penjimatan protein oleh sagu sebagai sumber karbohidrat. Di dalam Percubaan 1, empat diet isoproteik (50% CP) dan isolipidik (10% CL) telah dirumuskan menggunakan empat sumber karbohidrat yang berbeza (kanji ubi kayu, kanji jagung, kanji sagu dan dextrin) pada tahap kemasukkan yang sama (20%) dan diberi makan kepada kumpulan triplikat TGGG (13.78 ± 0.08 g purata berat awal). Ikan dipelihara dalam sistem air laut mengalir dan diberi makan dengan tangan dua kali sehari dengan diet percubaan hingga kenyang selama 67 hari. Pada penghujung percubaan makanan, berat badan diperolehi (BWG), kadar pertumbuhan spesifik (SGR), dan nisbah penukaran makanan (FCR) diet berasaskan kanji-kanji termasuk Diet Sagu (masing-masing 798.01%, 3.28%/hari dan 1.19), Jagung (masing-masing 782.09%, 3.25%/hari dan 1.17), dan Ubi kayu (masing-masing 780.90%, 3.24%/hari dan 1.15) tidak terjejas dan menunjukkan prestasi yang sama baik. Walau bagaimanapun, BWG dan SGR diperhatikan lebih rendah secara signifikan pada TGGG yang diberi makan Diet Dextrin (707.66%, 3.12%/hari) dan penemuan ini berkait rapat dengan pengambilan makanan (FI) kerana pengurangan yang ketara dalam pengambilan makanan diperhatikan dalam Diet Dextrin. Kemandirian hidup TGGG adalah 100% dalam semua rawatan tanpa perbezaan yang bererti (P>0.05) dikesan. Berbeza dengan prestasi pertumbuhan, indeks hepatosomatik (HSI) dan indeks viscerasomatic (VSI) jauh lebih tinggi dalam Diet Dextrin daripada diet berasaskan kanji. Kelembapan, abu dan lipid seluruh badan dipengaruhi oleh rawatan pemakanan. Walau bagaimanapun, protein tidak bergantung kepada sumber karbohidrat diet. Glukosa dan jumlah protein (P<0.05) telah terjejas dengan ketara oleh sumber karbohidrat diet dan tidak terdapat perbezaan yang ketara dalam kandungan kolesterol dan trigliserida di kalangan rawatan. Sebagai kesimpulan, semua kanji yang diuji boleh digunakan dengan jayanya sebagai sumber karbohidrat dalam diet untuk TGGG. Dalam percubaan 2, lima diet eksperimen dirumus menjadi isolipidik (10% CL) dan kanji sagu digunakan sebagai sumber karbohidrat tunggal. Dua tahap protein (40 & 45% protein mentah) telah diuji pada dua tahap karbohidrat (25 & 30% karbohidrat) (dikodkan sebagai 45P 25C, 45P 30C, 40P 25C, 40P 30C) dan Diet dengan 50% protein dan kanji 20% telah dirumuskan sebagai diet kawalan. Makanan ini diberikan dengan tangan kepada kumpulan triplikat TGGG (12.12 ± 0.08 g purata berat awal) selama 57 hari. BWG (576.62-618.91%), FCR (1.48-1.59), kemandirian hidup (93.33-

98.33%), dan indeks badan kecuali indeks viscerasomatik adalah tidak bergantung kepada rawatan diet yang berlainan. Sementara itu, komposisi proksimat seluruh-tubuh ikan, hati dan otot dipengaruhi oleh tahap protein dan karbohidrat yang berlainan. Koefisien penghadaman nyata termasuk bahan kering, protein dan lipid juga sangat dipengaruhi oleh diet yang diuji. Penemuan mencadangkan bahawa kanji sagu telah menunjukkan keupayaan penjimatan protein di mana peningkatan kadar karbohidrat sehingga 30% dan pengurangan protein kepada 40% dapat diterima oleh TGGG. Kajian ini telah menyumbangkan pengetahuan berharga kepada industri makanan akuakultur berkenaan sumber karbohidrat yang berbeza yang boleh digunakan dalam diet tanpa menjejaskan prestasi ikan dan potensinya dalam mengurangkan penggunaan protein mahal dalam diet.

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^{O}C	Degree Celcius
mg/kg	Milligram/Kilogram
%	Percentage
w/w	Wet/Weight
*U*m	Micrometre
Cm	Centimeter
m	Meter
mm	Milimeter
KJ/g	Kilojoule
mg/L	Miligram/Liter
L/min	Liter/Minute
g/L	Gram/Liter
mmol/L	Milimoles/Liter
fl	Fluid Ounce
mg/gm	Milligram/Gram
mM	Milimetre
g/kg	Gram/Kilogram
et al.	And Others
Sp.	Species

LIST OF ABBREVIATIONS

ADC	Apparent Digestibility Coefficients
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BWG	Body Weight Gain
CF	Condition Factor
CL	Crude Lipid
CP	Crude Protein
DHA	Docosahexaenoic Acid
DM	Dry Mass
EAA	Amino Acids
EPA	Eicasapentaenoic Acid
FAO	Food of Agriculture of United Nation
FBW	Final Body Weight
FCR	Feed Conversion Ratio
FI	Feed Intake
HB	Haemoglobin
HSI	Hepatosomatic Index
HUFA	Unsaturated Fatty Acids
IPF	Intraperitoneal Index
LRFF	Live Reef Fish Food
MCH	Mean Cell Haemoglobin
MCHC	Mean Cell Haemoglobin Concentration
MCV	Mean Cell Volume
MT	Metric Tonnes
NRC	National Research Council
PCV	Packed Cell Volume
PER	Protein Efficiency Ratio
PWG	Percentage Weight Gain
RBC	Red Blood Cell
RCC	Red Cell Count
SGR	Specific Growth Rate
SPSS	Statistical Package For The Social Sciences
TGGG	Tiger Grouper X Giant Grouper
US	United State
VSI	Viscerasomatic Index
WCC	White Cell Count

CHAPTER 1

GENERAL INTRODUCTION

1.1 Grouper Farming

The total world fishery production (capture and aquaculture) is expanding and expected to reach 196 million tonnes in 2025 (FAO, 2016). Globally, the impressive growth of aquaculture production has shown in all continents including the Asia-Pacific regions and expected to continue its contribution (FAO, 2015, 2016). The main target in aquaculture production especially in Asia Pacific Region is culturing high value marine species such as grouper (*Epinephelus* spp.). Grouper farming was introduced more than over 20 years ago in most part of the Southeast Asia using wild caught seed for grow out culture in cage, pond or pen farming. The demand for grouper is increasing thus promoting more grouper production from aquaculture. In 2015, total grouper production in Asia was 1114, 135.55 tonnes (FISHSTATJ). According to Annual Fisheries Statistics 2016 of Department of Fisheries Malaysia, the total aquaculture production of grouper hatchling and fry produced by government hatcheries were 2,296,100. Meanwhile, higher grouper hatchling and fry production were recorded in private hatcheries with the total of 115,100,000 and 21,649,458, respectively.

Grouper is one of the popular marine fish that widely traded in the Asia-pacific region, especially in Hong Kong and Southern China (Laining *et al.,* 2003). Since 2006, the live grouper is demanded either in the form of seeds for cage culture or table fish for seafood restaurant. Grouper species such as malabar grouper *(Epinephelus malabaricus)*, orange-spotted grouper *(E. coiodes)*, brown-marbled grouper *(E. fuscoguttatus)*, red-spotted grouper *(E. sexfasciatus),* and honeycomb grouper *(E. merra)* are the favorable species for grow-out culture in Malaysia, Singapore, Thailand, and Hong Kong. Although grouper farming is continuously to expand, the seed supply from wild and hatchery is still limited and poor survival of larvae makes it difficult to maintain the development of this sector (Rimmer, 2004). To sustain the growth of this sector, the hatcheries need to produce high quality grouper seeds in order to cope with the demand. Intensive research efforts have been done to encounter the problems by producing new species of desirable trait through hybridization, which help to sustain continuous seed supply of groupers and commercial farming.

Hybridization is the crossbreeding process between two species to produce a new species of desirable traits in offspring. The offspring produced has certain traits such as fast growth, better flesh quality and hardy offspring. Recently, there are several hybrid groupers that have been successfully produced and a hybrid between grouper, *E. fuscoguttatus* and *E. lanceolatus* (TGGG) has become one of the most sought after hybrids among consumers (Ch'ng and Senoo, 2008; Huang *et al.,* 2016).

Being relatively new aquaculture species, not many studies was conducted on the nutritional recommended of TGGG. In order to develop balance diets that can support good growth and survival of fish, nutritional requirement of the target species must be known. The nutritional requirement in fish is normally species specific. The basic components of formulated feeds are protein, lipid and carbohydrate. Protein and lipid are essential component in fish feed to meet up their requirement for essential amino acids and fatty acids. Carnivorous fish such as grouper need high protein level in the diet. However, the uncertain availability of fishmeal is the greatest challenges for sustainable fish farming. Thereby, understanding issues related to alternative ingredient that can minimize the use of fishmeal is critical.

1.2 Constraints in grouper farming

The grouper farming are facing constraint by high cost of feed (Pomeroy *et al.,* 2006). Since groupers are carnivorous fish that naturally demand for high dietary protein level, thus lead to the use of 'trash fish' in their feeding practices is common among the grouper farmers (Shapawi *et al.,* 2014). However, due to the many disadvantages caused by trash fish including inconsistent supply and assist in disease transmission to the culture area, the use of formulated feed in grouper farming had increased in the recent years (FAO, 2016). Historically, the common protein source has been fishmeal, produced from wild caught fish, while the lipid source has been the fish oil from the same source. Fishmeal production was declined since 2005 with continuous demand and fishmeal price are expected to remain high in the long term because of the growing demand in other industries as well (FAO, 2016). This has resulted in increased price for formulated feed (NRC, 1993; Usman *et al.,* 2007). Fish relies on dietary lipid and carbohydrate to spare protein that helps to reduce nitrogen wastes and archive feed formulations that do not compromise farmer's profit. By balancing the diets with non-protein ingredients, dietary protein can be lowered without impairing growth performance. In order to maintain consistent feed supply and aquaculture grouper industry, understanding issues related to alternative ingredients that can minimize the use of fishmeal is critical.

1.3 Importance of carbohydrate source in grouper diet

Carbohydrate is an organic compound which contains of carbon, hydrogen and oxygen in proportions. It is an excellent source of energy and carbon (Kamalam *et al.,* 2017). In general, carnivorous fish tend to utilize carbohydrate poorly, varying among species (Shiau and Lei, 1999). Fish do not have a true dietary carbohydrate requirement because of their ability to synthesize glucose from non-carbohydrate precursors such as lactate, pyruvate and amino acids (NRC, 2011). However, when fish fed diet without starch, they prefer to derive energy from the catabolism of protein for their metabolic function, but a substantial portion of protein can be spared for growth by optimizing the balance between protein and non-protein ingredients (lipid and carbohydrate) in diet (Li *et al.,* 2013). The optimum level of carbohydrate inclusion in fish diet can increase protein and lipid retention by preventing the catabolism of these expensive dietary nutrients for energy needs or sparing effect which help in reducing nitrogen load in the waste discharge, provide metabolites for biological syntheses, support feed formulations that maintain growth at a lower cost per unit gain, help in pellet binding, stability and floatability, and also facilitate the removal of feces through their binding properties (Hardy, 2010; Hemre *et al.,* 2002; Stone, 2003).

1.4 Source of carbohydrate

The classification of carbohydrate is dominantly based on chemical structure with commonly known groups being monosaccharides, disaccharides, and polysaccharides. Most of the plant-derived ingredients are in the form of energy reserves either in the form of simple sugar, polysaccharides and non-polysaccharides (NRC, 2011). However, only simple sugar and starch that have value in fish nutrition (Kaushik, 2001; Stone, 2003). The non-starch polysaccharides are known to have a negative nutritional role in fish (Sinha *et al.,* 2011). Starch is the main storage polysaccharides in plants that stored in the seeds, roots and fibers of plants. It is widely being used in food, feed and other industrial applications due to its binding properties. The chemical composition of starch varies with the source, which naturally have two different structure, amylose and amylopectin (Hua and Bureau, 2009; Mutungi *et al.,* 2012; Sweedman *et al.,* 2013). The starch can undergo certain process such as hydrolysis of starch catalyzed by hydrochloric acid and result a higher molecular weight polysaccharides, which called as dextrin.

1.5 Problem statement

Protein is one of the most expensive ingredients in formulated diet for fish. High dietary protein content is commonly associated with expensive feed cost and the negative environmental impacts due to the potential nitrogenous waste (Moreira *et al.,* 2008). Therefore, the interest to look for an alternative non-protein ingredient that can help to reducing dietary protein levels and maximizing protein utilization by considering the economical issues and environmental perspectives are crucial.

1.6 Significance of study

The inclusion of non-protein ingredient such carbohydrate offers the least expensive ingredient than lipid. However, the limited ability in carbohydrate utilization of marine fishes, such as in groupers are well known. Owing to this weakness, the knowledge in carbohydrate utilization including the suitable source or levels in groupers is important. Carbohydrate inclusion in fish feed should be considered due to its ability of protein sparing effect that can help to formulate cost-effective diet, so that the maximum profit was attainable which can be applied for sustainable aquaculture industry. Therefore, study on suitable carbohydrate source and optimum level that can be accepted in groupers is a fundamental aspect to be known before the optimum level of carbohydrate can be determined. The present study also focusing on locally available carbohydrate source as feed ingredients to reduce dependency on imported ingredients.

1.7 Hypothesis of study

The hypotheses of the study includes:

a) The tested carbohydrate sources (sago, corn, tapioca and dextrin) will be able to support good growth of TGGG

b) TGGG is expected to have some ability to spare protein with carbohydrate in the diet.

1.8 Objectives of study

The main aim of present study is to formulate feed for TGGG with the inclusion of suitable carbohydrate source as well as to optimized the level. Hence, there are two specific objectives in order to meet the overall objective of the study:

a) To determine the suitability of different carbohydrate sources in the formulated diets of TGGG

b) To determine the optimum level of protein and carbohydrate levels in formulated diets of TGGG

- In both of studies, the effects on growth performance, feed utilization, body indices, body composition, glycogen content, feed digestibility and blood parameter.

CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy, biology and distribution of groupers

There are 159 species of marine fishes with 15 genera. The 15 genera are *Aethaloperca, Alphestes, Anyperodon, Cephalopholis, Cromileptes, Dermafolepis, Epinephelus, Gonioplectrus, Gracila, Mycteroperca, Parathias, Plectropomus, Saloptia, Triso, and Variola.* Groupers are marine finfish belong to the subfamily Epinephelinae in the Order Perciformes, which are widely distributed in the tropical and sub-tropical waters of all oceans (Harikrishnan *et al.,* 2010). The species vary greatly but commonly have a wide body with a large head and mouth. The groupers are being identified by their colour pattern or suite morphological characteristics such as body shape, size and number of body parts (Govindaraju and Jayasankar, 2004). Groupers are solitary and sedentary fishes that usually found around the coral reefs, estuaries, rocky reef or habitat over a wide depth range (1-300 m) (Chiappone *et al.,* 2000; Dennis, 2015; Kandula *et al.,* 2015). Although grouper can be found mainly in open waters, their fry are found in tidal pool as well as coastal lagoon. The habitat of grouper depends on their life stages, the shallow water areas of coral rubbles (where the young fish settle), the coral reef (where the adults live), and the spawing aggregation sites (where adult gather to reproduce). Most groupers are protogynous hermaphrodites or they reproduce first as female and change sex later to reproduce as male (Erisman *et al.,* 2010). They are ubiquitous predator fish that feeds on small fishes, crustacean and cephalopods (Unsworth *et al.,* 2007). Groupers are also suboptimal fish and need optimum temperature between 24 to 30^{o}C, but they can survive at temperature 15 and 35^{o}C (Jiang, 2010).

TGGG grouper is a new species from cross breeding between tiger grouper (*E. fuscoguttatus, ♀)* X giant grouper *(E. lanceolatus, ♂)* was first produced in 2007 at the Borneo Marine Research Institute of Universiti Malaysia Sabah (Ch'ng and Senoo, 2008). Tiger groper (*E. fuscoguttatus*) as a female parent is also known as brown marbled grouper or dusky grouper. The body pattern is pale yellowish-brown and covered with large, irregular, dark brown blotches. The head, back and sides also covered with many tiny brown spots, and moderately deep bodied (Heemstra and Randall, 1993). They can grow up to 35kg and the maximum length can be up to 120cm (Tupper and Sheriff, 2008). Tiger grouper also can cost up to US$12-24/kg in Malaysia (Ch'ng and Senoo, 2008). The giant grouper (*E. lanceolatus)* as the male parent is also known as brindle bass, brindled grouper or Queensland grouper. It is one of the largest bony fishes and grows up to 3 m long and can weights up to 600 kg. They has robust body, with irregular broad black bars on body in small juvenile and small adult fish has irregular white or yellow spots on the black areas and fin with irregular spots (Heemstra and Randall, 1993). The giant grouper can cost up to US $90/kg in Malaysia (Ch'ng and Senoo, 2008). In aquaculture production, the farmers facing difficulties to culture both of this species where in tiger grouper, it is difficult to obtain high quality sperm while in giant grouper, it is difficult to obtain high quality eggs (Othman *et al.,* 2015). Thus, efforts have been made by researchers to solve the issues by improving the seed production through cross breed between these fish (Ch'ng and Senoo, 2008). Since then, TGGG become an important fish species in aquaculture industry and globally accepted especially in Hong Kong market. This hybrid has many advantages including fast growth, better tolerance to environment and resilience to disease than its parental species (Mustafa *et al.,* 2013; Liang *et al.,* 2013; De *et al.,* 2014, 2016; Othman *et al.,* 2015). Morphologically, TGGG seems similar to both of their parental species (Figure 2.1) (Fitriyani *et al.,* 2015).