Faculty of Resource Science and Technology

Protein Sparing Effect of Sago Starch in Diet of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758)

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Protein Sparing Effect of Sago Starch in Diet of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758)

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ABSTRACT

Six isoenergetic (20.25±1.35 kJ/g) semi-purified experimental diets were formulated with three levels of protein, P(22%, 26% and 30% diet) and two levels of sago starch as source of carbohydrates, C(38% and 44% diet). A corn starch (C40%) diet was used as a control diet. Diets were fed to triplicate groups of tilapia (n=20), *Oreochromis niloticus* juveniles (mean weight 4.61 ± 0.04 g), to apparent satiation twice daily for 12 weeks. The results showed 100% survival rate for all diet groups in which fish fed on 38% sago starch diet incorporated with protein level, P22% (D1: 29.53 g), P26% (D2: 31.87 g) and P30% (D3: 32.32 g) were significantly higher ($p < 0.05$) on final body weight (FBW) than those fed on 44% diet treatments combined with P22% (D4: 23.29 g), P26% (D5: 25.65 g) and P30% (D6: 27.71 g). Whole body proximate compositions (n=6) were significantly ($p < 0.05$) affected by 38% and 44% of dietary sago starch with different level of protein, P22%, P26% and P30, respectively. Fish fed on 38% sago starch diets with protein level, P26% (D2: 13.64 mmol/L) and P30% (D3: 13.68 mmol/L) showed high significant different ($p < 0.05$) of glucose concentration in plasma (n=6) than those fish fed on diet treatments with protein level, P22%, P26% and P30% which contained 44% of sago starch. Significantly higher ($p < 0.05$) of triglyceride plasma (n=6) were observed in fish fed on 44% sago starch with protein level, P22% (D4: 6.52 mmol/L) compare with those fish from other diet treatments, meanwhile, total protein plasma (p < 0.05) were varied from 4.18 to 22.11 mmol/L. Nutrients digestibility (n=12) showed significantly ($p < 0.05$) high statistics on fish fed with ratio P30%:C38% (D3:80.12%), followed by P26%:C38% (D2:77.54%), P26%:C38% (D1:74.72%), P30%:C40% (D0:69.83%), P30%:C44% (D6:65.67%), P30%:C44% (D5:57.40%) and P30%:C44% (D4: 50.29%), respectively. Digestive enzyme activities (n=12) were significantly ($p < 0.05$) affected among all diets in which fish fed on treatment

D3 with P30%:C38% ratio were statistically higher (amylase: 6.54 Umg^{-1}, lipase: 5.68 Umg^{-1}, protease: 0.77 Umg^{-1}) compare with the other diet treatments. The carbohydrate metabolic enzyme activities (n=15) showed significant ($p < 0.05$) influenced between 38% and 44% sago starch incorporated with protein level, P22%, P26% and P30% recorded approximately from 0.76 to 0.97 Umg^{-1} (PFK), 0.73 to 0.96 Umg^{-1} (FBPase) and 0.78 to 0.95 Umg^{-1} (G6PD). Two-way ANOVA result confirmed that the interaction between different level of both protein and carbohydrate have significant ($p < 0.05$) influenced on growth performance, whole-body proximate compositions, blood plasma compositions, nutrient digestibility digestive enzyme and carbohydrate metabolic enzyme activities of *O. niloticus* juveniles. Overall, fish fed on 38% sago starch-based diets showed positive result and performed better than those fed with 44% diets. The study revealed the ability of *O. niloticus* juveniles to spare protein by dietary carbohydrate was at optimum level of 38% sago starch and 30% protein.

Keywords: Sago starch, tilapia, growth, blood plasma, digestibility, digestive enzymes

Kesan Pengurangan Protein Menggunakan Kanji Sagu dalam Diet Tilapia Nila,
Oreochromis niloticus

ABSTRAK

Enam diet eksperimen separa tulen (20.25 ± 1.35 kJ/g) dirumuskan dengan tiga tahap protein iaitu P(22%, 26% dan 30% diet) dan dua tahap kanji sagu sebagai sumber karbohidrat iaitu C(38% dan 44% diet). Manakala itu, kanji jagung (C40%) pula digunakan sebagai diet kawalan. Tiga kumpulan juvenil ikan tilapia (n=20), Oreochromis niloticus (purata berat badan, 4.61 ± 0.04 g) diberi makan sehingga kenyang menggunakan diet tersebut iaitu sebanyak dua kali sehari selama 12 minggu. Hasil kajian menunjukkan kadar kelangsungan hidup adalah 100% untuk semua kumpulan diet di mana ikan yang diberi makan kanji sagu 38% dengan gabungan tahap protein, P22% (D1: 29.53 g), P26% (D2: 31.87 g) dan P30% (D3: 32.32 g) menunjukkan jumlah berat badan akhir (BBA) yang lebih tinggi ($p < 0.05$) daripada ikan yang diberi diet 44% yang digabungkan dengan P22% (D4: 23.29 g), P26% (D5: 25.65 g) dan P30% (D6: 27.71 g). Komposisi keseluruhan badan (n=6) adalah ketara ($p < 0.05$) iaitu dipengaruhi oleh gabungan antara 38% dan 44% kanji sagu dengan tahap protein yang berbeza iaitu P22%, P26% dan P30%. Ikan yang diberi makan kanji sagu 38% dengan tahap protein, P26% (D2: 13.64 mmol/L) dan P30% (D3: 13.68 mmol/L) menunjukkan kepekatan glukosa (n=6) yang lebih tinggi ($p < 0.05$) dalam plasma berbanding dengan ikan yang diberi diet dengan tahap protein, P22%, P26% dan P30% yang mengandungi 44% kanji sagu. Signifikasi yang lebih tinggi ($p < 0.05$) pada plasma trigliserida (n=6) diperhatikan pada ikan yang diberi 44% kanji sagu dengan kadar protein, P22% (D4: 6.52 mmol/L) berbanding dengan ikan dari kumpulan diet yang lain manakala jumlah protein plasma bervariasi dari 4.18 hingga 22.11 mmol/L. Pencernaan nutrien (n=12) menunjukkan statistik signifikasi yang lebih tinggi ($p < 0.05$) pada ikan yang diberi

diet dengan nisbah P30%:C38% (D3:80.12%), diikuti dengan P26%:C38% (D2:77.54%), P26%:C38% (D1:74.72%), P30%:C40% (D0:69.83%), P30%:C44% (D6:65.67%), P30%:C44% (D5:57.40%) dan P30%:C44% (D4:50.29%) juga sebaliknya. Aktiviti enzim pencernaan (n=12) adalah ketara ($p < 0.05$) di antara semua diet di mana ikan yang diberi diet D3 dengan nisbah P30%: C38% mempunyai kadar statistik yang lebih tinggi (amilase: 6.54 Umg^{-1}, lipase: 5.68 Umg^{-1}, protease: 0.77 Umg $^{-1}$) jika dibandingkan dengan kumpulan diet yang lain. Aktiviti enzim metabolik karbohidrat (n=15) menunjukkan kesan ketara ($p < 0.05$) iaitu dipengaruhi oleh gabungan antara 38% dan 44% kanji sagu dengan P22%, P26% dan P30% tahap protein iaitu mempunyai rekod anggaran dari 0.76 hingga 0.97 Umg^{-1} (PFK), 0.73 hingga 0.96 Umg^{-1} (FBPase) dan 0.78 hingga 0.95 Umg^{-1} (G6PD). Hasil daripada ANOVA dua hala mengesahkan bahawa interaksi antara tahap protein dan karbohidrat yang berlainan mempunyai kesan yang ketara ($p < 0.05$) terhadap prestasi pertumbuhan, komposisi badan, komposisi plasma darah, penghadaman nutrien, aktiviti enzim pencernaan dan enzim metabolik karbohidrat bagi juvenil O. niloticus. Secara keseluruhannya, ikan yang diberi makan diet 38% kanji sagu menunjukkan kesan positif dan prestasi yang baik berbanding kumpulan ikan yang diberi makan diet 44%. Kajian ini juga menunjukkan kemampuan juvana O. niloticus untuk mengurangkan protein melalui sumber karbohidrat pada tahap optimum adalah dengan menggunakan 38% kanji sagu dan 30% protein.

Kata kunci: *Kanji sagu, tilapia, pertumbuhan, plasma darah, penghadaman, enzim*

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LIST OF ABBREVIATIONS

FBPase	Fructose 1, 6-bisphosphatase
FBW	Final Body Weight
FCR	Feed Conversion Ratio
FI	Feed Intake
GE	Gross Energy
G6PD	Glucose 6-phosphate dehydrogenase
HSI	Hepatosomatic Index
IBW	Initial Body Weight
IPF	Intraperitoneal Fat
NFE	Nitrogen Free Extract
PER	Protein Efficiency Ratio
PFK	6-phosphofructokinase
SGR	Specific Growth Rate
SR	Survival Rate
VSI	Viscerosomatic Index
WG	Weight Gain

CHAPTER 1

INTRODUCTION

1.1 Study Background

The increases of global population growth have boosted the consumption demand of the fish as source of protein. Therefore, the role of aquaculture sector is important and an excellent approach to be an opportunity to bridge the supply and fill up the demand gap (Xu et al., 2019). Aquaculture is well known as the fastest growing food production sector in the world and produce a variety of products, hence reducing the pressure on the capture fisheries industry (Ng & Romano, 2013; Sandrea et al., 2017). It is also developing, expanding and intensifying in almost all regions of the world. The persistent goal of new world aquaculture is maximizing the efficiency of fish production and optimizing the profitability. However, the expansion of aquaculture to meet the rising demand for food supply increases the demand for more feeds from aquaculture feed industry, especially for intensive fish culture (Zhou et al., 2011; Fortes-Silva & Sànchez-Vàzquez, 2012).

Tilapia is an omnivorous fish of the family Cichlidae (Sklan et al., 2004; Qiang et al., 2014) which includes over 100 species under three genera; *Oreochromis, Sarotherodon* and *Tilapia* (Ridha, 2006; Wang & Lu, 2016). Tilapia is the most cultured fish in the world and has been recognized as the great economic important for many countries. Currently, tilapia is the second most farmed fish in the world after carps, with global production estimated around 6.3 million tonnes in 2018 (FAO, 2019) and expected to reach 7.3 million tonnes by 2030 (FAO, 2013). The global tilapia market is valued about 11,700 million USD in 2017 and estimated to be reach 13,400 million USD by the end of 2025 which is mounting at a CAGR of 1.8% during the period. Nile tilapia (*Oreochromis niloticus*) is the most farmed among the tilapia species (Singh et al., 2006; El-Sayed & Kawanna, 2007) and have greater demand for larvae and juveniles (Ribeiro et al., 2018).

Global aquaculture production was 114.5 million tonnes in 2018 and was predicted to reach 109 million tonnes in 2030, an increase of 32% over the period (FAO, 2019). In aquaculture feed industry, protein is one of the most expensive and important nutrient for fish growth. In view of this, studies on the quality and optimal requirement of protein in feed formulation for *O. niloticus* are important to reduce feed production costs. Moreover, feeds containing high level of protein do not necessary indicate better growth but depend on the bioavailability of the amino acids (Gao et al., 2011). This might due to the ability of fish to utilize protein which specifically related to the existence of proteases in the digestive system that are responsible for hydrolyzing or breaking down the protein into peptides and amino acid and eventually absorbed by fish for growth (Luo et al., 2016).

In aquaculture field, the successful and sustainable are depends on the provision of nutritionally balanced, environment friendly and economically viable artificial feeds. Generally, the feed preparation diet is a principal factor that increase the growth and production of reared fish in aquaculture (Thankur et al., 2004; Liti et al., 2005; Abdel-Tawwab et al., 2007). However, the high cost and short supply of protein source in feed production is the main problem facing by majority aquaculture industry nowadays (Sayed et al., 2018). This also may become the biggest challenge to formulate more effective feeds which is very important for fish in order to reach maximum growth rate and balanced energy requirements. Consequently, the alternative and strategic approach for the issue is important because the aim is not only to reduce cost but also to make feeds become more adequate and sustainable.

The main protein source used in aquaculture feed industry is fishmeal. This is due to its high quality of protein, stable amino acids profile, essential fatty acids content, high digestibility and better palatability to fish (Wang et al., 2017). FAO (2019) reported that it was estimated about 70% of total fishmeal production was utilized for aquaculture feeds. However, due to low production and consistent increase in demand, the price of fish meal rose to USD 1,650 per tonne in 2018, the highest from the previous years (FAO, 2019). This has forced the aquaculture feed manufacturers to use cheaper alternative protein source as fishmeal replacement to sustain the developing aquaculture production. Fishmeal is commercially made from the whole fish like chapelin, herring, anchovy or menhaden white fish including the bones and offal after lipid extraction (Wu et al., 2015). Generally, the nutrient compositions of fishmeal were comprised of approximately between 66 to 71% protein, 5 to 9% lipid, 7 to 8% moisture and 12 to 19% ash (He et al., 2015; Rahmah et al., 2016).

Indeed, the total production cost have been the main issue in fish feed manufacturing. This due to dietary protein source which is considered as the main and most expensive ingredients in fish diet (Boonanuntanasarn et al., 2018). Therefore, the alternative approach is to spare the protein source in the aquaculture feed with less expensive ingredients such as lipids and carbohydrates. In the present study, the protein-sparing effect of sago starch in the diet for Nile tilapia, *O. niloticus* juveniles was conducted. Sago starch is extracted from sago palm, *Metroxylon sagu* which is also considered as a potential candidate for carbohydrate source to spare protein in aquaculture feeds production (Umar et al., 2013). To take into account, the largest supply of sago comes from South East Asia, particularly Indonesia and Malaysia (Jong, 2018). In Sarawak, the sago production mainly occurred in Mukah and it is a popular foodstuff especially among the Melanau ethnic group in the area (Karim et al., 2008).

1.2 Problem Statement

The cost-effective development and formulation of adequate nutritional diet is important in any reared fish species. In the fish culture, practical feeds are the important aspect for source of nutrients and also the main factor which greatly influence the total yield of fish in the aquaculture farm. However, feed is often considered as one of the major challenges for aquaculture practices, as it represents the main contributor to operational costs approximately between 40 to 60% production expenses of the whole farm (Azaza et al., 2015). Therefore, the feed must be economical and sustainable besides nutritionally sufficient relevance with the demand of current aquaculture industry (Watanabe, 2002; Deng et al., 2010).

Protein is very crucial macronutrient in aquafeed as it provides major energy source, build and repair the damaged tissues and for general body maintenance. It is an essential nutrient and well known as the most expensive component in fish diet (Zhang et al., 2016). The optimum level of protein used in fish diet is crucial to prevent the amino acid from catabolized as energy rather than growth (Stone et al., 2003a). It is reported that the catabolism of protein in the diet can be minimize for energy metabolism through the adequate levels of non-protein energy sources such lipid and carbohydrate (Mohanta et al., 2007; Kamalam et al., 2017). Thus, the use of another alternative sources with reliable nutrient contents such as carbohydrate source that can reduce feed costs is important to make the fish culture become more effective and economic (Boonanuntanasarn et al., 2018).

Dietary carbohydrates are considered as less expensive energy source component in aquatic animal feeds and efficiently utilized by omnivorous and/or herbivorous warm water fish (Wang et al., 2014; Kong et al., 2019). Starch is the predominant carbohydrate in plant such as wheat, corn, field peas, grains and legumes and have been incorporated into formulated diets for fish (Lee et al., 2003). In addition, sago (*Metroxylon sagu*) starch also may considered as a nutritive and potential carbohydrates sources for aquaculture feeds (Umar et al., 2013). The application of carbohydrates in fish diets have a great benefit in aquaculture sustainability because it contributes to low cost feed formulation, increases protein retention, reduces ammonia excretion, and improves stability and floatability of pellets in extruded diets (Wang et al., 2016; Corrêia et al., 2019). However, the use of carbohydrate as a protein-sparing energy source in fish has received less attention compared to lipid while the utilization of digestible energy in the form of carbohydrate is considered as a prime importance under practical aquaculture conditions as stated by Mohanta et al. (2009).

Furthermore, a clear knowledge and better understanding on the optimal level of protein as dietary carbohydrates used is important to study the protein-sparing effect that may be essential in minimizing the cost of fish preparation diets (Abdel-Tawwab et al., 2010; Rahman et al., 2017). Boonanuntanasarn et al. (2018) mentioned that the application of dietary carbohydrate as a protein-sparing source of energy still showed fewer attention in formulated feed for cultured fish. Besides, there is little information available on the growth performance, blood plasma, nutrient digestibility, digestive and carbohydrate metabolic enzymes response in fish that utilized on starch-based carbohydrate diets (Qiang et al., 2014).

1.3 Objectives

This present research was designed to evaluate the effect of Sago (*Metroxylon Sagu*) starch in the diet for Nile tilapia, *Oreochromis niloticus* juveniles. Specifically, the objectives were:

i. To determine the protein-sparing capacity of sago starch in promoting tilapia, *Oreochromis niloticus* juveniles growth performance.

ii. To investigate the nutrient digestibility of sago starch based diets in *Oreochromis niloticus* juveniles.

iii. To determine the digestive and carbohydrate metabolic enzymes of the fish in response toward the sago starch.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview

This section provides the information on comprehensive knowledge of previous research on the present study. Briefly, this chapter explained on challenges in aquaculture, details on *O. niloticus* (taxonomy, distribution, biology, economic importance, feeding mechanism, digestibility enzymes) and also the potential of sago starch as source of dietary carbohydrate in fish formulated diet. These will be explained further in the following subsections.

2.2 Global Aquaculture and Challenges

Aquaculture is the culture of aquatic organisms, which includes fish, mollusks, crustaceans, algae and plants (Chen et al., 2018). Aquaculture has a long history whose root can be traced back for more than 2000 years in China (Allen & Steeby, 2011; Azaza et al., 2015). The intensification of aquaculture and globalization of the seafood trade have led to remarkable developments in the aquaculture industry (Boonanuntanasarn et al., 2018). Aquaculture is the fastest growing food-producing sector in the world. It is developing, expanding and intensifying in almost all regions of the world. Since the global population is increasing, thus, the demand for aquatic food products are also increasing. Recently, the production from capture fisheries have level off and most of the main fishing areas have reached their maximum potential (FAO, 2019). Therefore, sustaining the fish supplies from capture fisheries are no longer able to meet the growing global demand for aquatic food. Thus, aquaculture is considered as a good potential to be an opportunity to bridge the supply and demand gap of aquatic food in most regions of the world.

The principal challenge of global aquaculture tends to mitigate the supply and demand of fish and fisheries products in paralleled with the outbreaking population growth in the world. The FAO (2019) reported that the total aquaculture production including the aquatic plants for 2018 was 114.5 million tons, where only fish and fisheries aquaculture production was 82.1 million tons with a farm-gate value USD 263.6 billion. This inland aquaculture production was accounted at 62.5% contribution in total fisheries production (Figure 2.1). The State of World Fisheries and Aquaculture (FAO, 2019) reported that the world food fish aquaculture production was at 46% in 2018 which was increased at an average annual rate of 25.7% in the period of 2000 to 2018. During this period, the growths were relatively faster in Africa (17.9%), followed by Europe (17%), Americas (15.7%) and Oceania (12.7%). It was reported that the share of aquaculture in Asian fish production (excluding China) has reached 42.0% in 2018, up from 19.3% in 2000 (FAO, 2019). China has produced more farmed aquatic food than the rest of the world combined since 1991. Since the introduced of government policies in 2016, fish farming in China grew by only 2.2% and 1.6% in 2017 and 2018, respectively. China's share in world aquaculture production has declined from 59.9% in 1995 to 57.9% in 2018 and is expected to decrease further in the coming years. Nevertheless, comparison to the projected population by 2030, an additional 40 million tons of fish and fisheries production will be required to maintain the present per capita consumption. Therefore, this sector is going to face some challenges, which are already adopted. Presently, aquaculture is thought to be the fastest growing food producing sector and is perceived as having the greatest potential to meet the growing demand for aquatic food.