UNIVERSITI PUTRA MALAYSIA

EFFICACY OF STEVIOL GLYCOSIDE SUPPLEMENTATION ON SURVIVAL GROWTH AND BLOOD BIOCHEMISTRY PROFILES OF HYBRID RED TILAPIA (*Oreochromis* sp.)

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By

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Steviol glycoside is a healthy low calorie natural sweetener extracted from a bushy plant, *Stevia rebaudiana* Bertoni which is used in the human food industry. Despite extensive research conducted to investigate the properties of steviol glycoside for human consumption, only a few reports is available on its use in aquafeeds. This research aimed to investigate the potential of steviol glycoside in the diet of the fish by measuring the survival, growth index, gut short chain fatty acid and blood plasma biochemistry. Tilapia were fed diets prepared by two different methods: extrusion and compact pelletization. The effects of supplementation of 0, 1, 3 and 6% of dietary steviol glycoside on the survival, growth, blood plasma chemistry and short chain fatty acid of red tilapia were investigated. Both experiments showed that stevia had no significant effects on fish growth, proximate and short chain fatty acid composition. However, an apparent sweet smelling taste was observed while manufacturing the feeds using a common pelletizer. In addition, increasing dietary steviol glycoside level in feed made with a common pelletizer decreased hepatosomatic index (HIS) indicating stress while extruded feeds with increasing amount of dietary steviol glycoside resulted in higher viserosomatic index (VSI) indicating more fat was deposited in the viscera. Further plasma analysis between stevia-free and 6% steviol glycoside fed fish showed no significant differences in blood plasma glucose, total protein, cholesterol and triglycerides. In conclusion, dietary steviol glycoside did not give any beneficial effect to hybrid red tilapia. Inclusion of 6%, in fact, indicated stress based on the decrease in HSI and irregular changes in blood plasma biochemistry of glucose, total protein, triglycerides and cholesterol trend.

Key words: Steviol glycosides, tilapia, growth, plasma chemistry, short chain fatty acid

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

KEBERKESANAN PENAMBAHAN STEVIOL GLIKOSIDA PADA KEMANDIRIAN, PERTUMBUHAN, DAN PROFIL BIOKIMIA DARAH TILAPIA MERAH (*Oreochromis* sp.)

Oleh

JASON CHOY MIN SHENG

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Steviol glikosida adalah pemanis semulajadi berkalori rendah yang diekstrak daripada tumbuhan renek *Stevia rebaudiana* Bertoni yang digunakan dalam industri makanan manusia. Walaupun banyak penyelidikan telah dijalankan untuk menyiasat sifat steviol glikosida bagi penggunaan manusia, hanya terdapat sebilangan kecil laporan yang menyiasat penggunaannya dalam makanan akuakultur. Kajian ini bertujuan untuk mengkaji potensi steviol glycoside dalam diet ikan dengan mengukur kemandirian, indeks pertumbuhan, asid lemak rantai pendek dan biokimia plasma darah. Tilapia diberi makan diet yang disediakan menggunakan dua kaedah; ekstrusi dan pemampatan. Kesan penambahan 0, 1, 3 dan 6% steviol glikosida dalam diet terhadap kemandirian, pertumbuhan, kimia plasma darah dan asid lemak rantaian pendek dalam tilapia merah telah dikaji. Kedua-dua eksperimen menunjukkan tiada kesan ketara stevia terhadap pertumbuhan, dan komposisi proksimat dan asid lemak rantaian pendek. Walau bagaimanapun, bau manis jelas diperhatikan ketika penghasilan pelet menggunakan penguntil biasa. Di samping itu, peningkatkan kandungan steviol glikosida dalam diet yang diproses menggunakan penguntil biasa menurunkan kadar hepatosomatik (HSI) yang menunjukkan stres/tekanan sementara peningkatan steviol glikosida yang diproses menggunakan pengekstrusi menghasilkan kadar viserosomatik (VSI) yang lebih tinggi menunjukkan lebih banyak lemak disimpan dalam visera. Seterusnya analisis plasma antara ikan yang diberi diet tanpa dan 6% steviol glukosida menunjukkan tiada perbezaan yang ketara dalam aras plasma glukosa, protein keseluruhan, kolesterol dan trigliserida. Sebagai kesimpulan, penggunaan steviol glikosida tidak memberi sebarang kesan positif kepada tilapia merah. Penambahan 6% steviol glikosida dalam diet sebaliknya memberi stres kepada tilapia merah berdasarkan penurunan kadar HSI dan

perubahan abnormal dalam biokimia plasma darah seperti perubahan trend glukosa, jumlah protein, trigliserida dan kolesterol.

Kata kunci: Steviol glikosida, tilapia, pertumbuhan, biokimia plasma, asid lemak rantaian pendek

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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 Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
g	gram
cm	centimeter
DFI	daily feed intake
FCR	feed conversion ratio
SGR	specific growth rate
HSI	hepatosomatic index
VSI	viscerosomatic index
SCFA	short chain fatty acids
PER	protein efficiency ratio

CHAPTER 1

INTRODUCTION

Tilapia is the second most cultured fish family in the world after a group of carp species with a total production of almost 4,200 million tonnes in year 2016 (FAO, 2018). Similarly, tilapia is also the second most farmed freshwater fish in Malaysia after African catfish. Its production was 25,648 tonnes with the market value of over RM 250 million in year 2017 (DOF, 2018).

Formulating a nutritionally complete and balanced feed is important as it will be the only food the organism will be consuming throughout the entire culture period. Formulated aquafeeds must satisfy all the dietary nutrient and energy requirements of the culture species. General consideration for feed formulation of tilapia includes protein, lipids, fiber, ash, methionine, vitamins and minerals contents. Protein and lipid are the most expensive ingredients in formulated diets and thus have been extensively studied for tilapia but not carbohydrate.

Research in fish utilization of carbohydrate is intensifying due to the growing use of plant based proteins as an alternative to fishmeal. Carbohydrate metabolism of fish is not well understood, including for tilapia, but it can have a substantial effect on the development of fish (Ng & Romano, 2013). In general, fish tend to utilize more complex sugars such as dextrin, disaccharides and starch compared to simple sugars. While tilapia may not be particularly able to digest carbohydrate efficiently, it may be fermented by intestinal bacteria in the fish gut producing short chain volatile fatty acids (SCFA), increasing energy availability to the fish and creating a less ideal environment for intestinal pathogens.

Among dietary carbohydrates, there is limited information on the effects of stevia on the growth and metabolism of fish. Stevia, a natural sugar substitute and sweetener, is extracted from the plant *Stevia rebaudiana*. The active compound in the sweetener is steviol glycoside. Stevia has been used by the natives of Paraguay and Brazil since pre-recorded history (Ahmed et al., 2011) and subsequently became known in 1887 due to the “discovery” of stevia by a botanist, Antonio Bertoni. Stevia later was particularly noted in Japan where it was banned on 1970’s as a food ingredient in beverages. Through much effort and deliberation by various food regulatory agencies, stevia was later approved by the US Food and Drug Administration (FDA) in 2010 and is currently and slowly being globally reintroduced into the market after satisfying various safety requirements. Post approval, stevia proven to contain many intrinsic therapeutic benefits for the human diet such anti-diarrhoeal, anti-hyperglycemic, anti-inflammatory, anti-hypertensive, anti-

tumour, diuretic and immunomodulatory effects (Chatsudthipong & Muanprasat, 2009).

1.1 Problem Statement

The various benefits of stevia are well documented in human via first using rats as a model to understand the specific pathways resulting to a deeper understand of the biology of rats and ultimately humans. However, there is no such initiative to understand the effects of stevia on fish. It may be possible that stevia can improve feed intake and nutrient utilization and therefore better growth in a cost-effective manner. However, this has not yet been demonstrated on fish.

1.2 Objectives

This research aimed to determine whether dietary steviol glycoside could act as a growth promoter or feed attractant that might improve feed utilization and efficiency among tilapia. The effects of incorporating varying levels of steviol glycoside in two types of feeds were evaluated. The effectiveness of steviol glycoside was assessed through growth performance, feed utilization, hepatosomatic index (HSI), viscerosomatic index (VSI), plasma biochemistry (total protein, glucose, triglycerides and cholesterol) and SCFA levels of the fish. The results might be useful to the fish feed industry as to whether to include steviol glycoside as one of feed ingredients in manufacturing high quality fish pellet.

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Jason Choy Min Sheng was born on 27 November 1988 in Penang, Malaysia. He graduated with a Diploma in Fisheries and Bachelor of Science in Agrotechnology majoring in Aquaculture from Universiti Malaysia Terengganu in 2009 and 2012, respectively. He was on a Malaysian Government Scholarship for his bachelor education. He underwent an internship at Research Institute for Aquaculture No. 1 in Hanoi, Vietnam in 2011. He worked briefly as a graduate assistance in Universiti Sains Malaysia, Penang under the guidance of Prof. Dr. Ng Wing Keong and finally landed a job as a lecturer at Jerantut Community College, Malaysia. He is always passionate about the aquaculture industry and has interest to formulate better feed for the industry. After three years of lecturing and exposure in the industry, he decided to further his education by enrolling in Master of Science program at the Universiti Putra Malaysia by part time mode since he was unable to secure a study leave based on eligibility. He thanks SEARCA for providing a scholarship for his M.Sc. study.

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