EFFECTS OF *Chlorella vulgaris* ENRICHED *Artemia* sp. AND *Moina* sp. TO ENHANCE GROWTH PERFORMANCE OF KELAH, *Tor tambroides* (BLEEKER, 1854) FRY

By

WIZILLA JANTI ANAK JOSHUA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

I would like to dedicate to this dissertation to myself – thank you for hanging on. To my parents, siblings and partner who are always supportive of my ambitions and nurturing me with endless affection and love, you all inspire me to be a better version of myself every day.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Tor tambroides is a species valued in Malaysian aquaculture but faces limitations in its potential due to slow growth. This study was designed to investigate the effects of *Chlorella vulgaris* enriched *Artemia* sp. and *Moina* sp. to enhance growth performance of Kelah (*Tor tambroides*) fry. The objectives of this study were to determine the nutritional profile of *Chlorella* sp. as potential candidate for live food enrichment; to evaluate and compare the composition of unenriched and *C. vulgaris* enriched *Artemia* sp. and *Moina* sp.; and to assess on the effects of *C. vulgaris* enriched *Artemia* sp. and *Moina* sp. in comparison to unenriched counterparts on the growth performance, survival and expression of growth and immune genes of *Tor tambroides* fry. The identification of *C. vulgaris* targeting 18S rRNA gene resulted nucleotide sequence of *Chlorella* sp. with 99.72% to 100% matches with *C. vulgaris*. The obtained lipid content was 3.29%, with high PUFA content (67.04%). In the second study, *Artemia* sp. and *Moina* sp. were enriched with *C. vulgaris*. The two-way ANOVA revealed that the type of livefood (*Artemia and Moina*) and unenrichment versus enrichment had significantly influenced the moisture and ash content ($p<0.05$). The enriched *Artemia* had the highest lipid content (25.68%) and it was statistically different as compared to the unenriched and enriched *Moina* ($p<0.05$). The linoleic acid was the highest in enriched *Moina* (12.19%). The total PUFA was the highest in unenriched *Artemia* (41.61%), followed by enriched *Artemia* (39.90%) but both treatments were not significant to one another ($p>0.05$). In the third study, the fries were divided into four diet treatments; unenriched *Artemia* (UA); enriched *Artemia* (EA); unenriched *Moina* (UM); enriched *Moina* (EM). The growth parameters were significantly higher in fries group fed with enriched *Artemia* ($p<0.05$). The variations of the growth genes suggested that there were differences in growth rate in the fry groups, meanwhile the expression of MSTN was proposed to be related to other physiological processes. The immune response genes showed similar pattern expressions in enriched *Artemia*, suggesting its immunocompetency values. In conclusion, enrichment of *Artemia* with *C. vulgaris* enhances the growth performance and expressions of growth and immune response genes, making it a promising diet for slow-

growing species like *Tor tambroides.*

Keywords: *Artemia; Chlorella vulgaris;* enrichment; *Moina; Tor tambroides*

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

PENGARUH *Artemia* sp. DAN *Moina* sp. DIPERKAYAKAN DENGAN *Chlorella vulgaris* DALAM MENINGKATKAN PRESTASI PERTUMBUHAN BENIH KELAH, *Tor tambroides* (BLEEKER, 1854)

Oleh

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Tor tambroides adalah ikan yang bernilai tinggi dalam akuakultur Malaysia. Spesies ini menghadapi cabaran disebabkan pertumbuhan yang perlahan. Kajian ini fokus pada penggunaan *Artemia* sp. dan *Moina* sp. yang diperkaya dengan *Chlorella vulgaris* untuk meningkatkan prestasi pertumbuhan anak ikan Kelah *(Tor tambroides*). Objektif kajian merangkumi mengenal pasti profil nutrisi *Chlorella* sp. sebagai calon untuk pengayaan makanan hidup, membanding komposisi *Artemia* sp. dan *Moina* sp. yang diperkaya dan tidak diperkaya dengan *C. vulgaris*, serta menilai kesan pada pertumbuhan, ketahanan, dan ekspresi gen pertumbuhan dan imun anak ikan *Tor tambroides*. Pengenalpastian *C. vulgaris* melibatkan gen 18S rRNA, menunjukkan padanan nukleotida 99.72% hingga 100%, dengan *C. vulgaris*. Kandungan lipid yang diperoleh adalah 3.29%, dan terdapat 22 asid lemak dengan kandungan PUFA yang tinggi (67.04%). Dalam kajian kedua, Artemia sp. dan *Moina* sp. masing-masing diperkayakan dengan *C. vulgaris* selama 6 jam dan 24 jam. Analisis ANOVA dua hala menunjukkan bahawa jenis makanan hidup dan pengayaan mempengaruhi kandungan lembapan dan abu secara signifikan ($p<0.05$). *Artemia* yang diperkaya menunjukkan kandungan lipid tertinggi (25.68%), berbeza secara statistik berbanding dengan *Moina* ($p<0.05$). Asid linoleik tertinggi terdapat dalam *Moina* yang diperkaya (12.19%). Kandungan PUFA tertinggi dalam *Artemia* yang tidak diperkaya (41.61%), diikuti oleh *Artemia* yang diperkaya (39.90%), tanpa perbezaan signifikan antara keduanya ($p>0.05$). Dalam kajian ketiga, anak ikan dibahagikan kepada empat rawatan makanan; *Artemia* yang tidak diperkaya (UA); *Artemia* yang diperkaya (EA); *Moina* yang tidak diperkaya (UM); *Moina* yang diperkaya (EM). Parameter pertumbuhan menunjukkan prestasi yang lebih tinggi secara signifikan dalam kumpulan anak ikan yang diberi *Artemia* yang diperkaya ($p<0.05$). Varian gen pertumbuhan menunjukkan perbezaan dalam kadar pertumbuhan di kalangan kumpulan anak ikan, sementara ekspresi MSTN dikemukakan berkaitan dengan proses fisiologi lain. Gen respons imun menunjukkan corak ekspresi yang serupa dalam *Artemia* yang diperkaya, menunjukkan nilai imunokompeten. Kesimpulannya, pengayaan *Artemia* dengan *C. vulgaris* meningkatkan prestasi pertumbuhan dan ekspresi gen pertumbuhan

dan respons imun, menjadikannya diet yang berpotensi untuk spesies yang pertumbuhannya perlahan seperti *Tor tambroides*.

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

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LIST OF ABBREVIATIONS

ALA	α-linolenic acid
BLAST	Basic Local Alignment Search Tool
bp	Base pairs
CC3	Complementary C3
DAH	Days after hatching
˚C	Degree Celsius
DOF	Department of Fisheries
DNA	Deoxyribonucleic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAO	Food and Agriculture Organization
g	Grams
GH	Growth hormone
hr	Hour
kg	Kilogram
LNA	Linoleic acid
L	Liter
LC-PUFA	Long-chain polyunsaturated fatty acid
mRNA	Messenger ribonucleic acid
MHCC1a	Major Histocompatibility Complex Class 1a
µL	Microliter
µm	Micrometer

mg	Milligram
mL	Milliliter
mm	Millimeter
mm^2	Millimeter square
MUFA	Monounsaturated fatty acid
MSTN	Myostatin
nm	Nanometer
%	Percentage
PCR	Polymerase chain reaction
PUFA	Polyunsaturated fatty acid
rpm	Revolutions per minute
RNA	Ribonucleic acid
RM	Ringgit Malaysia
SFA	Saturated fatty acid

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The freshwater aquaculture is defined as the rearing and breeding of aquatic organisms in captivity which include fish, shrimp, shellfish and aquatic plants for commercial aims (Li et al., 2018). Typically, species selected for aquaculture are those with high and fast growth and tolerant to extensive environmental conditions (Julian et al., 2021). Based on the data by FAO (2020), global aquaculture production in 2018 was 48% and out of that, freshwater aquaculture had contributed to 60% of the total production (Keller and Lodge, 2021). Due to such rapid expansion of freshwater aquaculture, numerous of developments in fish nutrition and feed competency have been highlighted with the direction towards sustainability. This is also related to the feeding regimes and suitable feed during the early stage of larviculture, which in turn will support the optimal growth and higher survival of the larvae (Rasdi et al. 2019; Lee, 2003).

In Malaysia, *Tor tambroides* is one of the fish species with aquaculture potential. It is a riverine cyprinid with high value in Southeast Asia (Lau et al., 2021). It is treasured and highly regard as an important ornamental and sport fish with stable and high market value (Azfar-Ismail et al., 2020). According to Department of Fisheries (DOF), the wholesale value of this species could reach up to RM 233.37 per kilogram and had the production of 105,904.01 tonnes in 2021. Thus, making it a great potential in aquaculture industry (Ingram et al., 2005). Various efforts have been taken to further understand this species in different aspects, including its breeding performance (Ingram et al., 2007; Ingram et al., 2005), molecular systematics (Esa et al., 2008), morphometric analysis (Pollar et al., 2007), ontogenic development (Ramezani-Fard et al., 2011) and its environmental water parameters in the farms (Soon et al., 2014).

1.2 Problem statement

Unlike other fast-growing species in aquaculture, *T. tambroides* is a slow grower fish as compared to other cyprinids (Asaduzzaman et al., 2018). It may take a year to achieve 500 g to 600 g body weight in its natural environment (Ng, 2004). Several researches had emerged in order to enhance the knowledge on the nutrition requirements of this fish species at various life stages. Among the studies focusing on its dietary requirements during the fingerling stage were the effects of the dietary protein requirement and the lack of protein-sparing action by dietary lipid on its growth performance based on semi-purified diets and different levels of proteins and lipids (Ng et al., 2008), effects of commercial poultry offal meal replacing fishmeal on its growth performance (Ismail et al., 2013) and effects of different starch sources on its growth, body composition and feed intake efficiency (Ishak et al., 2021). Meanwhile, the nutritional studies during the juvenile stage were highlighting on the effects of different dietary lipid percentage over time of feeding on its muscle fatty acid compositions

(Ramezani-Fard et al., 2012) and the effects of crude illipe (*Shorea macrophylla*) oil on its growth performance, body composition and fatty acid profile (Kamarudin et al., 2018). The earliest study on the larvae stage of *T. tambroides* were done by Asaduzzaman et al. (2016) through the feeding with live and formulated feeds (65% fishmeal, 2% squid meal and 2% shrimp meal) and their impacts on the biochemical composition and growth performance of the larvae, whereby the formulated feed had demonstrated better results than live foods. Although the formulated feed was tested in T. *tambroides* larvae (Asaduzzaman et al., 2016), it might not be cost effective and sustainable for early larval feeding as it includes high inclusion of fishmeal (65%).

1.3 Justification of study

The development of larviculture and hatchery are deemed as the critical parts in aquaculture. It is known as a bottleneck in seed production for aquaculture (Herath and Atapaththu, 2013). In regard to hatchery management and operation is the use of live food as the diets of fry. Generally, live foods are more desirable as compared to artificial feeds during the early fish and crustacean larval stage (Das et al., 2007). The dependency on live food in the hatcheries are expected to be continuously applied in the future (Dhont et al., 2013). The digestive enzymes are found in abundance in live foods and it stimulates larval appetite (Zeng et al., 2018). Among the commonly used live food sources in the hatcheries for the culture of both marine and freshwater larvae and fry are *Artemia* and *Moina*. These live foods are known as the filter feeders. They have the ability to filter any compound available in the water column (Sorgeloos et al., 2001; Manklinniam et al., 2018). Such characteristics in *Artemia* and *Moina* are utilized to deliver certain nutritional contents through enrichment technique to target species.

Microalgae possess high nutritional values such as long chain polyunsaturated fatty acid (LC-PUFA) which constitute of phospholipids (Li et al, 2018; Li and Olsen, 2015). The green microalgae, *Chlorella vulgaris* is a good candidate for enrichment of live food. It is known as the super food with various nutrients such as fats, protein, vitamins, bioactive compounds and carbohydrates (Andrade et al., 2018). It contains immunostimulant properties which is beneficial in enhancing the lifespan of fish (Gouveia et al., 2002) and boost the growth of several fish species including Olive flounder (*Paralichthys olivaceus*) (Rahimnejad et al., 2017), koi carp (*Cyprinus carpio*) (Khani et al., 2017) and African catfish (*Clarias gariepinus*).

Besides, the administration of enriched live food, particularly *Artemia* sp. and *Moina* sp. enriched with *C. vulgaris* is yet to be discovered in *T. tambroides* fry focusing on the growth performance, survival and expression of growth and immune response genes. The long culture period of *T. tambroides* in captive condition is contributed by the fact that it is a slow-growing fish (Chowdhury et al., 2016; Soon et al., 2014). This drawback could impact the seeds availability and the period of this species to reach the marketable size which indirectly hinder its potential in aquaculture industry. The growth and immune systems are related to the interaction and metabolism of nutrients (Martin and Król, 2017; Moriyama et al., 2001; Peter and Marchant, 1995). Studies supported that *Artemia* and *Moina* enriched with various enrichment media had resulted in positive effects in terms of growth performance and survival as compared to the unenriched counterparts. For instance, *Artemia* enriched with *Chlorella* sp. had resulted in higher survival of juvenile long snout seahorse, *Hippocampus guttulatus* (Palma et al., 2011), whereas *Chlorella* sp. enriched *Moina* had contributed to the high specific growth rate and survival in Siamese fighting fish (*Betta splendens*) juvenile (Rasdi et al., 2020b). These promising effects could be based on species specific as different larval species may be affected differently to the enrichment diets and it does not always contribute to positive effects. For example, *Artemia* enriched with vitamin A did not give any significant effects to the growth performance and survival of strip trumpeter (*Latris lineata*) (Negm et al., 2014). The *C. vulgaris* enriched *Artemia* and *Moina* could be potential candidates for *T. tambroides* fry during weaning period before artificial and formulated feed are being offered as the sole diet and this could possibly enhance its growth performance and survival during early stage of lifecycle.

1.4 Objectives

Hence, the objectives of this study were:

1. To screen and analyze of the nutritional profile of *Chlorella* sp. as potential candidate for live food enrichment
2. To evaluate and compare the composition of *Artemia* sp. and *Moina* sp. enriched and without enrichment of *C. vulgaris*
3. To assess on the effects of *C. vulgaris* enriched *Artemia* sp. and *Moina* sp. in comparison to unenriched counterparts on the growth performance, survival and expression of growth and immune genes of *Tor tambroides* fry.

Hypothesis:

H0: The C. *vulgaris* enriched *Artemia* sp. and *Moina* sp. does not affect the growth performance, survival and expression of immune and growth genes of *Tor tambroides* fry

H1: The C. *vulgaris* enriched *Artemia* sp. and *Moina* sp. does affect the growth performance, survival and expression of immune and growth genes of *Tor tambroides* fry

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