DEVELOPMENT OF AQUACULTURE FEED FOR *Oxyeleotris marmorata* USING *Hermetia illucens PRE*-PUPAE BIOMASS

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DEVELOPMENT OF AQUACULTURE FEED FOR *Oxyeleotris marmorata* USING *Hermetia illucens* PRE-PUPAE BIOMASS

By

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ABSTRACT

DEVELOPMENT OF AQUACULTURE FEED FOR *OXYELEOTRIS MARMORATA* USING *HERMETIA ILLCUENS* PRE-PUPAE BIOMASS

Chin Yik Chun

Hermetia illucens larvae are well known to be nutritious as a substitute for fish meal in aquaculture due of their high fat and protein content, insect larvae meal can be used as an alternative protein source to fish meal, which will lower production costs and the need for raw materials while also minimizing a number of environmental problems. Hence, this study was aimed to investigate the growth performance of marble goby (*Oxyeleotris marmorata)* fed with substitute fish oil source obtained from black-soldier fly (*Hermetia illucens*) at different composition levels. The pre-pupae of *Hermetia illucens* had sufficient amount of crude lipid (46.83 ± 1.37%) and crude protein (43.84 ± 1.08%) to exhibit healthy growth. The juvenile marble goby was fed with formulated fish diet of HM0, HM40, HM80 and HM120 for 12 weeks containing 15.49- 16.10 MJ/kg of energy, and 45.06 – 47.98% and 9.57 – 10.26% of crude protein and crude lipid content, respectively. The juvenile gained 1.01% to 25.72%, in weight with feed conversion rate of 15.27 to 188.52 and protein efficiency of 0.011 g weight gained/ g protein to 0.137 g weight gained/ g protein. The survival rates displayed by the juvenile were 40% to 80%. Comparatively, the juvenile fed on feed with HM40 exhibited the best result in terms of both

survival rate of 60.0% and weight gained (17.03%) while retaining 49.06 ± 0.07% of ΣSFA, 27.35 ± 0.12% of ΣPUFA and 23.59 ± 0.09% of ΣMUFA. In overall, the *Oxyeleotris marmorata* fed with diet HM40 (4% inclusion level of *Hermetia illucens* pre-pupae meal) showed the best growth performance throughout experimental feeding trial.

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APPROVAL SHEET

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It is hereby certified that **Chin Yik Chun** (ID No: **18AGM06463**) has completed this dissertation entitled "DEVELOPMENT OF AQUACULTURE FEED FOR *Oxyeleotris marmorata* USING *Hermetia illucens* PRE-PUPAE BIOMASS" under the supervision of Dr. Leong Siew Yoong (Supervisor) from the Department of Petrochemical Engineering, Faculty of Engineering and Green Technology, Dr. Tey Lai Hock (Co-Supervisor) from the Department of Chemical Science, Faculty of Science and Dr. Loo Joo Ling from Department of Mechatronics and BioMedical Engineering, Lee Kong Chian Faculty of Engineering and Science.

I understand that University will upload softcopy of my dissertation in pdf format into UTAR Institutional Repository, which may be made accessible to UTAR community and public.

Yours truly,

_____

(Chin Yik Chun)

DECLARATION

I, CHIN YIK CHUN hereby declare that the dissertation is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

(CHIN YIK CHUN)

Date: 30/04/2023

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Symbols/Abbreviations	
ACC	Acetyl-CoA carboxylase
ACIAR	Australian Centre for International Agricultural Research
AOCS	American Oil Chemist's Society
AOAC	Association of Official Agricultural Chemists
ARA	Arachidonic acid
AV	Acid value
BSF	Black soldier fly
CASIP	Cultured Aquatic Species Information Programme
CF	Crude fibre
CL	Crude lipid
cm	Centimeter
CMC	Carboxylmethyl cellulose
CP	Crude protein
CuSO4	Copper(II) sulphate
DHA	Docosahexaenoic acid
DM	Dry matter
DoF	Department of Fisheries
e.g.	Exempli gratia (for example)
EPA	Eicosapentaenoic acid
et al.	Et alia (and others)
FA	Fatty acid

FAME	Fatty acid methyl esters
FAS	Fatty acid synthase
FCR	Feed conversion ratio
FMOC	Fluorenylmethyl chloroformate
g	Gram
GC – FID	Gas Chromatography – Flame Ionization Detector
GDH	Glutamate dehydrogenase
GDP	Gross domestic product
gI_2	Gram of iodine
GLA	γ-linoleic acid
g/day	Gram per day
HI	*Hermetia illucens*
HM	*Hermetia illucens* meal
i.e.	Id est (that is)
IFFO	International Fishmeal and Fish Oil Organization
HCl	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
HSI	Hepatosomatic index
H_3BO_3	Boric acid
H_3PO_4	Phosphoric Acid
IV	Iodine value
JH	Juvenile hormone
JPSPN	Jabatan Pengurusan Sisa Pepejal Negara
kg	Kilogram
K_2SO_4	Potassium sulphate

L	Litre
LA	Linoleic acid
M	Molarity
MAFF	Ministry of Agriculture, Forestry and Fisheries
MeOH • H_2O	Methanol–water mixture
mEq	Milliequivalents
MG	Marble goby
mgKOH	Microgram of potassium hydroxide
min	minute
MJ	mega-joules
mL	millilitre
mol	molecule
MPA	3-mercaptopropionic acid
MT	Metric tonnes
MSU	Michigan State University
MUFA	Monounsaturated Fatty Acids
N	Normality
NaH_2PO_4 • H_2O	Sodium dihydrogen phosphate monohydrate
NaOH	Sodium hydroxide
NFE	Nitrogen-free extract
OA	Oleic acid
OECD	Organization for Economic Co-operation and Development
OPA	Orthophthalaldehyde
PER	Protein efficiency ratio

PES	Polyethersulfone
PTFE	Polytetrafluoroethylene
PTTH	Prothoracicotropic hormone
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
RAS	Re-circulation aquaculture system
RM	Ringgit Malaysia
SEAFDEC	Southeast Asian Fisheries Development Center
SFA	Saturated fatty acid
SGR	Specific growth rate
SGRW	Specific growth rate based on weight gained
SGV	Sand goby virus
SOD	Superoxide dismutase
pH	Potential hydrogen
USD	United States dollar
UTM	University Technology Malaysia
VSI	Viscerosomatic index
wt	Weight
20E	20-hydroxyecdysone
μL	microlitre
μmol	Micro-molecule
%	Percent
ºC	Degree Celsius
Σ	Summation

CHAPTER 1

INTRODUCTION

1.1 Introduction

Aquaculture in general refers to the practice of breading and rearing fish in controlled clean environment in culture systems in order to provide source of food to a growing world population (Rozana, 2020). Aquaculture farming has few varieties which includes but not limited to the fresh-water farming, marine-culture and alga-culture. The use of the natural environment such as lakes, river and ponds as culture systems instead of aquarium tanks are widely utilized for more intensive and high productive farming. 50% of world sea-food supply is grown by the aquaculture industry (Bush, et al., 2013). Aquaculture organism output increased at an annual rate of 5.3% from 2001 to 2018 (FAO, 2020). Whereby, finfish dominated the aquaculture production in 2018 at around 54.3 million tonnes. (Food and Agriculture Organization of United Nations, 2020). It is estimated that 24 million employments is sustained by the aquaculture industry. In recent years, consumption of protein source from aquaculture had an increase of 4.7% from 20.4 kg in 2017-2019, and is estimated to be at least 21.4 kg by 2029 (OECD/FAO, 2021). The demand for fish as a protein source especially produce from the aqua-farms is expected to account for 62% of total worldwide production by 2030, as the world's population continues to grow. Aquaculture demand accounted for 46% of total

output in 2018, and with current favourable developments, it may be feasible to significantly expand in the next years (Ahmad, et al, 2021).

Malaysia has been and still is a maritime nation through and through. Placed safely in a rich environment both land-wise and also marine wise has always been a boosting drive for the aquaculture in Malaysia. The government has been always pushing for improvement and growth of the sector for the sake of developing the nation. With a growing population and a growing desire for fish as a healthy source of animal protein minerals and fatty acids (FAO, 2016). In terms of fish production, in 2018 it is recorded to have reached 1.84 million ton, 21% was product of aquaculture while 79% was capture fisheries. The high cost of the production due to limited resources, limited supply of input and also the chances of infectious diseases might be reason for the downward trend. In addition, the backlash received from the environmental agencies on the impacts of aquaculture which have irreversibly disrupted and diminishing the ecosystems has raised considerable concerns. Thus, in order to move forward, the aquaculture not only has to keep up to the demand but also have to be sustainable for the environment.

Malaysians consume more fish as compared to any other protein source at per capita intake of fish of at least 56.5 kg per year, which is considered the highest in the South-East Asia (Hicks, et al., 2019). This amounts to approximately 60% to 70% of the total national protein intake (Jeevanaraj, et al., 2019). In terms of technology, farmed species, and contribution to national income, Malaysian aquaculture has advanced dramatically during the last 15

decades where the progress has been closely and vastly linked to the domestic and international demand (Kurniawan, et al., 2021). Brackish water fish, freshwater fish, shrimps, prawns, ornamental fish and aquatic plant being just the fraction of commodities produced in Malaysian aquaculture industries both governmental (102.1 million ton fish produced) and private (1872.5 million ton fish produced) employs up to 49,989 fish farmers and culturists in 2019 (Department of Fisheries Malaysia, 2020). According to FAO (2020), black tiger shrimp and white leg shrimp was the most lucrative brackish water aquaculture. Meanwhile, for freshwater, red tilapia and catfish are considered the most valuable species (FAO, 2021).

The wild populations of aquatic life have over the decade taken full brunt of over-fishing. With lesser and lesser fish landing, guaranteed food supply will continue to be a major challenge in the future. Cultivation of fish in farms were once considered environmentally friendly, instead it has been a huge polluter of aquatic environment so far (Findlay, et al., 1995). Aquaculture has mostly been linked with water pollution where cage culture causes anoxic sediment, disruption of biodiversity at the water bed, and cause of destructive eutrophication (FAO, 2015). Meanwhile, aquaculture is also to be blamed for the deforestation of millions of mangrove in order to expand as the signs are visible in countries like Thailand and Indonesia (Naylor, et al., 2000; Harper, et al., 2007). Besides that, the capturing of the fingerlings by the non-selective fishing gears, which are mostly comprised of nets with lower mesh sizes and in some circumstances, zero mesh sizes, are proven to be very damaging means of

seed collection, harmful toward the seedling and also toward the habitat itself (Mandal, 2021).

Aquaculture's proportion of worldwide fishmeal consumption increased dramatically from 10% in 1980 to 73% in 2016. However, global fishmeal production is estimated to be approximately 5 million metric tonnes (MT) per year, increasing steadily over the past 20 years, with future output influenced by rising demand, climate change, and unpredictability (e.g., El Niño) (Malcorps, et al., 2019; Hardy, 2010; Tacon and Metian 2008). Nearly 70% of farmed finfish output is reliant on artificial feeding rather than the natural environment for nutrition (FAO, 2018; Shannon and Waller, 2021; Cashion, Le Manach and Pauly, 2017). The application of fish oil due to rise in aquaculture has shifted from margarine and shortning production in 1960s to aqua-feed (over 70%) in 2010 (Shepherd and Jackson, 2013). According to IFFO report, despite the rise in demand for fish meal and fish oil, supply has remained unchanged (Shannon and Waller, 2021). This pattern can be said to be a direct result of decline in catches of fish. Subsequently, causing a decrease in fishmeal production of up to 1,000,000 metric tonnes (mt), especially in an El Niño year (Hardy, 2010). It is an undeniable fact that fishmeal and oil supplies are a finite source, and the aquafeed industry must look for alternative ingredients from other sources whose worldwide output is adequate to meet the industry's demands for the foreseeable future. Despite these advancements, the aquafeed industry's consumption of fishmeal has increased as aquaculture production has grown. As a result, further reductions in fishmeal percentages in aquafeed is required. Continued reductions in fishmeal and fish oil levels are expected for

some farmed fish species; full replacement of fishmeal has been achieved in laboratory experiments (Hardy, 2010).

Sustainable aquaculture seems like an impossible dream, with dozens of factors plaguing its sustainability; feed is considered one of those factors (Hardy, 2010). Alternatives must be further developed and used if aquaculture is to be sustainable in terms of feed input (Fisher, et al., 2020; Tacon, and Metian, 2015; Ytrestøyl, et al., 2015). The price of fishmeal and oil in comparison to alternative ingredients, as well as insufficient information on the nutritional requirements of major farmed species and the bioavailability of essential nutrients required to formulate feeds containing alternative ingredients, are the main drivers of change in aquafeed formulations (Hardy, 2010). The overall price of commercial aquafeed has remained constant due to the fact that use of alternative ingredients especially plant based ingredient has become mainstream (Turchini et al. 2019). Aquafeed ingredients are inclusive commodities with applications ranging from uses in livestock and poultry to applications in pet food (Malcorps, et al., 2019; Cashion, Le Manach and Pauly, 2017; Hardy, 2010). Currently, the majority of fishmeal is made up of whole fish, with just 25–33% made up of fisheries by-products or undesired discards, however this percentage is expected to climb in the future (Shepherd and Jackson, 2013; Shannon and Waller, 2021; Cashion, Le Manach and Pauly, 2017). It is worth noting that about 64% of the fishmeal and fish oil used to feed aquaculture species comes from fish (mostly forage fish) that are caught especially for this purpose, rather than by-products of fisheries (Shannon and Waller, 2021). Squid by-products (Abdul Kader et al., 2012), poultry and cattle sector by-products,

but most importantly plant oils and seeds, especially soya bean-based diets, are among the possible alternatives (Shannon and Waller, 2021; Wan, et al., 2018). Soy protein concentrate is the most common plant-based protein source, accounting for 19.2% of total diet ingredients, followed by wheat gluten (9.0%), corn gluten (3.4%), horse beans (2.0%), pea protein concentrate (1.4%), faba beans (1.3%), sunflower meal (1.2%), and other marginally used plant proteins (2.7%) (Aas et al., 2018). However, due to critical elements that are changeable or unbalanced in terrestrial plant components, certain aquatic species' nutritional requirements may restrict the quantity of fishmeal substitution (Malcorps, et al., 2019). Plant subsituent have a poor protein content, imbalanced essential amino acid profiles, low palatability, antinutrients, and rivalry from other food and feed industries (Krogdahl et al., 2010). Furthermore, replacing plant components for fishmeal would transfer resource demand from the seas to the land, thereby putting strain on land-based food production systems, impacting the ecosystem, biodiversity, and crop availability and pricing (Malcorps, et al., 2019).

Other than that, use of insect such as *Hermetia illucens* as substitute in aquafeed has gained momentum over these past few years due to the fact that insect meals are the most environmental, economic, and sustainable of all other alternatives (Arturo et al., 2018; Surendra et al., 2016). Insects as aquafeed have several advantages over conventional fish and plant protein, including the ability to be raised on organic waste (vegetable, fruit, factory waste, and meat waste) with little water input, high feed conversion efficiency, low emissions of carbon dioxide, methane and ammonia, fewer animal care issues, and a lower

risk of desease transmission (Yi, et al, 2020; van Huis, et al., 2013). *Hermetia illucens* in particular are of great interest since conventional mass-rearing procedures for industrial production of high-quality products already exist (Yi, et al, 2020; Wang and Shelomi, 2017; Henry et al., 2015; van Huis et al., 2013). *Hermetia illucens* meal (HM) depending on its rearing condition have wide yet stable amino acid profile with high crude protein (around 55% of dry weight) and crude lipid content (30% of dry weight basis), not to forget good source of minerals and vitamins (Xu, Zhang, Song, & Sun, 2014; Belghit, et al., 2018; Henry et al., 2015; Bußler et al., 2016; Liland et al., 2017; Wang and Shelomi, 2017; Newton et al., 1977).

Besides that, *Hermetia illucens* also contains high levels of polyunsaturated fatty acid (PUFA), increasing its overall quality making it perfect for omnivorous and carnivorous fish such as European-seabass and Artic salmon (Zhou, et al., 2017; Belghit et al., 2018; Hu et al., 2017; Li, et al., 2017; Sealey, et al., 2011; St-Hilaire, et al., 2007). Replacement of *Hermetia illucens* as a fishmeal in fish feed has been successfully carried out, however only to a certain extent (Dumas et al., 2018; Magalhães et al., 2017; Renna et al., 2017; Belghit et al., 2018 ; Elia et al., 2018). In regards to that, there is gross lack in research on the use of *Hermetia illucens* in feed for freshwater marble goby *(Oxyeleotris marmorata)* (Yong, et al. 2015). This South-East Asian fish costs an arm and yet its market value is still on the rise. Due to feeding behavior of marble goby which tend to reject most feeds, still have no commercially formulated feed available, making it one of, if not the most challenging fish to domesticate (Teoh, Lim, and Kawamura, 2018).

1.2 Problem Statements

Rising organic waste production is a concerning dilemma not only for Malaysia but for the whole world. Food waste accounts for around 61% out of 931 million tonnes in 2019 was generated from households (United Nations Environment Programme, 2021). Malaysian on the average wastes a staggering 16,688 tons of food on the day to day basis (Hashim, et al., 2021). In the past, the most effective way to manage food waste has been landfills. However, this practice is becoming more and more difficult as the existing landfills are almost fully filled up (Moh and Manaf, 2014), while furthur expansion are becoming more and more hard as more land are used to house and accommodate the rising population. Incineration on the other hand causes air pollution (Zhang, et al., 2014) and not to forget the effects the harmful greenhouse gasses on the climate. A more sustainable approach to this is by using the organic waste as feed for rearing insects. This approach reduces the use of land significantly while having almost non-exsisting carbon footprint. The reared insect can be futher used as insect meal substituting the conventional protein and lipid source to formulate appropriate animal feeds

Diminishing population of aquatic fish and increasing demand for fish as a protein source are causing supply shortages and rising of market fish prices are vividly seen (SEAFDEC, 2021). As the wild catches reduce day to day, nutritional cost for aquafeed or fishmeal in particular has become more expensive (El-Sayed, 2006; Webster and Chorn, 2006) and thus, an urgent need

for alternative protein source as fish meal which is more sustainable in term of land, cost and environment has risen. Aquafeed in most part has always been readily available commercially. With the fact that reducing number of wild aquatic organism, a more sustainable protein source may be the best way in order to increase not just the productivity but at the same time increase profitability. Looking back at the possible candidates, from livestock to plants, one candidate is shown to have the ability to substitute as a fishmeal. In moments of protein scarcity (Riggi, et al., 2013), insect has always been the best alternative due to the fact that insect possessing the ability to turn bio-waste into protein and fat. The idea of insect replacement as a protein source has been around since the early 1990's which now have become a mainstream (Calvert, Martin and Morgan, 1969; Ogunji, et al., 2006). *Hermetia illucens* is one of the candidates for the protein replacement as farming would have less carbon footprint and very less land is required for the farming.

Marble goby, *Oxyeleotris marmorata* is one of the most expensive freshwater fish in the market due to the high and rising demand for the fish. The demand in terms of export of the fish reaches as far east as-Japan. Meanwhile, the fish is a staple in lives of the local of South-East Asian countries. The popularity of the fish is associated with the texture and immaculate taste. The mass production of the fish is still out of reach and most feedings not surviving the early stages. Most of these issues are due to the feeding habits of *Oxyeleotris marmorata* which refuses most feeds. However, with the correct farming technique and suitable aquaculture feed, marble goby has the greatest potential

to be cultivated in farms especially now when there are dwindling population in the wild. Furthermore, It has been reported that marble goby is a lean fish which more likely to utilize monounsaturated fatty acids (MUFAs) for energy source and could have the ability in bio-converting linoleic acid (LA) to ARA (Bundit, 2007). It is hypothesized the existence of physiological needs of marble goby towards MUFAs and omega-6 (n-6) fatty acid for optimal performance. Therefore, the present study aimed to evaluate the effects of dietary fatty acids particularly the oleic acid (OA) to LA ratio on growth performance, body compositions,

1.3 Research Objectives

The objectives of this research are as follows:

i. To farm black soldier fly (*Hermetia illucens*) pre-pupae for development of aquafeed for *Oxyeleotris marmorata* at various ratio (0-100 of dry wt %).
ii. To study the effects of the developed aquafeed on the growth performance, body compositions and biochemical parameters of *Oxyeleotris marmorata*.
iii. To determine the content of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of *Oxyeleotris marmorata* after feeding with the developed aquafeed.

CHAPTER 2

LITERATURE REVIEW

2.1 The Economical and Sustainable Aquaculture Industry in Malaysia

Malaysia's rich aquatic ecosystems supply the locals with vital resources including freshwater, source of food and medicine while providing protection, and flood control. The ecosystem also provide the nation with the means of commercial aquatic resources, energy and transportation for economic and social development of its people. However, with the rising global ambient temperatures, rising sea water level, over-fishing and water pollution are dealing severe and irreversible damages to the diverse ecosystem.

Aquaculture industry had gained momentum in order to keep up with increasing demand for fish based protein. Malaysians consumes approximately 36 kg of fish per capita making up around 60 – 70% of protein requirement as fish is considered a healthier source of animal protein, a direct result of the the (Rahimah and Adinan, 1992; Mazlan, 2000; Hicks, et al., 2019; Jeevanaraj, et al., 2019). As of production, in 2018 alone Malaysia produced at around 395900 tonnes (Department of Fisheries Malaysia, 2019). More fish from farms are being eaten now compared to all the catches of the open sea, this is the crude reality of the situation and is projected to provide around 62 % of the global

demand by year 2030 while Asia is expected to consume 70% of the total production. Due to aquacultures potential as food security, has allowed the sector to make progressive achievements in genetics breeding programs (Allison, et al., 2011; Bentsen, et.al, 2017). Culture systems technologies (Norazmi, et al., 2017), aquaculture management practice (Dickson, et al., 2016), and aquaculture feed (Carrier, et al., 2017, Magalhaes, et al., 2017, Kurniawan, et al., 2021; Shannon and Waller, 2021) in order to efficiently increase its production.

Asia has been the biggest producer of fishery products both from capture and aquaculture; therefore it is considered the birthplace of aquaculture industry (SEAFDEC, 2021; FAO, 2016b; FAO, 2016c; Tacon, et al., 1995). Based on the Fishery Statistics Bulletin of Southeast Asia (SEAFDEC, 2021), the total production from inland capture fisheries in the region has continuously increased for this past few decades. The marine fisheries resource is the main supply of fisheries commodity in Malaysia. In 2018, the marine fisheries catch was 1,476.9 thousand tonnes, which rose by 0.8% against 1,465.1 thousand tonnes in 2017 (Department of Fisheries Malaysia, 2019). The development of the fisheries sector in Malaysia is expected to emerge as one of the major agricultural contributors to the national economy, both as a source of foreign exchange and, more importantly, as a source of animal protein. Over the last decade, employment in the fishing industry has increased (Shariff and Subasinghe, 1993). Aquaculture generates an estimated 10,995 jobs for Malaysians in 2017 acording to Department of Statistics Malaysia, accounting for around 8.9% of the entire national agricultural gross domestic product

(GDP). It indicates that the industry not only has to supply national food security but it functions as the potential contributor to relieving hunger and poverty across the world. Inland fisheries are one of the most economically significant sub-sectors at the local and national levels due to its contribution to food security and better earnings in rural areas, yet, because of the nature of inland fishing operations, which are mostly small-scale, highly seasonal, and primarily carried out by part-time fishermen, and a large portion of the production is intended for domestic consumption and not recorded at landing sites, information on production from inland capture fisheries in the region has always been very limited (SEAFDEC, 2021).

Aquaculture can be divided into onshore freshwater aquaculture which is said to have developed early on in Malaysia and the offshore marine or brackish aquaculture which soon followed by brackish aquaculture species in Malaysia are mostly finfish, custodian fish, bivalve and seaweed. Meanwhile, fresh-water inland of Malaysia has a total of around 300 recorded species of fish and around 200 are reported in peninsula in 1989 (Zakaria-Ismail, 1996). Pahang state has the highest diversity of freshwater species followed by Terengganu and Johor not far behind (Lee et al., 1993). It is believed that ex-mining pools is where aquaculture industry in Malaysia first had its humble beginning with cultivation of Chinese bighead, grass and silver carps which later expanded into other fish and shrimp farming (Iliyasu, et al., 2016). Semi-intense shrimp culture with marine fish culture did not begin until the early 1970s, followed by extensive commercial cultures in the 1990s (Yusoff, et al.,

2007).The country witnessed a source to table strategy to improve food safety and fish health management in the 2000s (Melba, et al., 2005). Freshwater fish, freshwater prawns, marine finfish, marine shrimp, and mollusks are the five primary aquaculture products listed in Malaysia's National Agro-Food Policy for the period 2011-2020, with an overall objective of 8.6% annual growth (Iliyasu, et al., 2016). In Malaysia, the marine fisheries resource is the primary source of fisheries commodities. In year 1983, there were 712 fish species, with 460 of them being economically significant (Mohsin and Ambak, 1996). Started from 2021-2030, as part of the National Agro-Food Policy 2.0, the government is considering aquaculture zoning, the development of new regions while boosting existing output, and improved local and export market share, all of which are supported by sound research, development and commercialization. Which fulfilled and complied with National Agro-Food Policy 2.0 objectives such smart and modernized agriculture; excessive productiona and market strengthening; development of human capital; sustainable food system and creation of conductive bussines ecosystem and governance (National Agrofood Policy 2021-2030).

Aquaculture consists several culture systems that can be employed in a farm all varying on species. One of the most common culture is pond which is suitable for both freshwater and seawater fish and also prawns. However, states like Perak, Johor and Selangor which have over 4300 abandoned tin mining pools and lakes can be used as a culture for freshwater fishes besides being used as water supply and waste disposal as reported in 1994 (Arumugam, 1994).

Cage culture is mainly reserved for marine fishes and was first employed in Malaysia in the early 1970's (Chua, 1979). As the population increase and suitable land becomes scarce, more farmers are opting for cage polyculture. Aquarium fish farming make use of small ponds and tanks in order to breed and cultivate fish and could be considered the most versatile.

Aquaculture production is often praised for its ability to produce nutritious seafood in a highly efficient manner (Klinger and Naylor, 2012; Sprague, et al., 2016), but is also often criticised for unsustainable production practices, especially concerning use of feed (Ytrestøyl, et al., 2015) and its negative impact on local environmental conditions (Klinger and Naylor, 2012; Osmundsen, et al., 2017). The public is increasingly aware that aquaculture carries environmental risks (Alexander, et al., 2016; Morton and Routledge, 2016; Olsen and Osmundsen, 2017) and that the seafood they consume may originate from unsustainable source. The successful development of this sector is dependent on our ability to exploit resources efficiently and sustain growth without adversely affecting the aquatic environment (Zakaria-Ismail, 1996). Sustainable herein is defined from the context of both the environment and its end users which eventually consume the food product (Lee, et al., 2016). Fishery stocks of inland waters in the country, as elsewhere in the world, have been under increasing pressure from other uses of land and water resources. The present trend is the increasing dominance of small-sized fish landings (Department of Fisheries Malaysia, 2000) due to intensive fishing efforts that even catch the small fish. Increasing pressure on land, deforestation of water

catchment areas, and conversion for lands to agriculture, has increased soil erosion.

In terms of its contribution to food security for the poor and disadvantaged groups of people dependent on the harvest of natural resources, inland capture fisheries serve as important sources of micronutrients and calcium requirements from small fishes the possible impacts of freshwater aquaculture on the environment, e.g. restriction of access to inland water bodies, discharge of effluents that contaminates natural aquatic habitats, spread of diseases and pathogens, culture of few commercial species impacting biodiversity and genetic diversity in natural habitats, introduction and contamination of non-indigenous or invasive species, should also be considered. Competition for the use of waters and areas could also happen between inland fishers and fish farmers (FAO, 2016; MSU, 2016).

The aquaculture sector in Malaysia faces significant difficulties and challenges as projected by the agriculture production from 2013 to 2018 which shows a downward trend by Agrofood Statistic (2018). The high cost of feed caused by the reducing fish population and the regulation put in place by the government are perceived to be hindering the growth of the aquaculture industry (Yue, et al., 2014; Fitzsimmons, et al., 2017) Around the world, around 30% of fisheries are overfished, more than 60% are fully fished, and less than 10 per cent have remaining in sufficient capacity (FAO, 2014). Diseases are also another factor slowing the growth of aquaculture. Outbreak of a disease such as

a case of the tilapia lake virus effects the fishes during Israel's warmer months of June to October where the temperature rises to about 20 degree Celsius (Fathi, et al., 2017) causing mortality rate spike to an alarming 90% (Eyngor, et al., 2014).

Pollution especially the plastic waste is irrefutably one of the main culprits for the depletion of the marine-life. It is believed that around 8 million ton of the plastic waste daily is littered into the vast blue ocean. The problem is real and the evidence is irrefutable as study conducted by examining the two locations offshore of Brazil's coastline found plastic waste in up to 22% of the landed fish stomach (Daniele, et al., 2016). The plastics wastes drifting in the open sea are usually mistaken as prey and thus ingested. Consequently, the scarcities of catches compared to previous decades are being felt all over the world.

In the past, the widespread and abundant supply of natural resources led in little concern about their conservation and its long-term use. Human activities such as exploitation of living resources, huge land reclamation and development, emission of hazardous industrial and domestic pollutants, and large energy development projects are all threatening these resources. Threats to the long-term viability of these resources, such as land-based and sea-based pollution, are growing more significant, affecting people's health and prosperity. Water shortages, dirty rivers and seas, a drop in fishery commodities, a decline in aesthetic qualities in water-based recreational places, and a decrease in

biodiversity are all signs of disrespect for the aquatic environment. Freshwater is considered a precious commodity which is around only 1 per cent of world's water found inland as bodies of lakes, rivers and underground reservoirs, a finite resource sustaining life on land. With the rising human population, sustainable freshwater management are of the utmost importance.

Modification, destruction, or complete loss of habitat: Among the coastal ecosystems, mangroves are the most greatly affected by aquaculture since most aquaculture ponds were constructed in mangrove areas. Southeast Asia has the widest and the most diverse mangroves in the world but between 1980 and 2005 it suffered a decline of 26.46% (Spalding et al., 2010). Most of these losses were due to conversion into milkfish and shrimp ponds (Naylor et al., 2000), resulting in loss of goods and ecosystem services generated by mangroves—plant and wood products, provision of nursery habitat, coastal protection, flood control, sediment trapping, and water treatment (Bandaranayake, 1998; Ewel, et al., 1998; Macnae, 1968). Aside from losing these goods and services, converting mangroves into aquaculture ponds transforms an open access fisheries with multiple users to a privatized farm resource of few wealthy individual investors and business enterprises.

2.2 *Oxyeleotris marmorata* and Its Market

Oxyeleotris marmorata (Bleeker, 1852) is known globally as marble goby (FAO,

2014). Other names commonly used are ‘soon hock’ (Singapore), ‘ikan ubi’ (Malaysia), ‘ikan ketutu’ (Malaysia), ‘bakut’ (Malaysia & Indonesia), ‘bakutut’ (Indonesia), ‘bia’ (Philiphines), ‘cá-bong’ (Vietnam), ‘Damrey’ (Cambodia), ‘kawanago’ (Japan). Marble goby belongs to the Eleotridae family is a tropical globoid fish which lives in both breakish and freshwater habitat. *Oxyeleotris marmorata (Bleeker)* is an amphibious fish capable of breathing air and thus able to survive outside of water for up to several days.

Marble goby is a natural predator residing close to deltas, ponds, lakes (Great Lake, Tonle Sap and Laguna De Bay), rivers (Chao Phraya River, Citaram River and Mekong River) and swamps (Roberts, 1993). In the wild, carnivorous marble goby naturally feeds on arthropods (shrimps, marine and raw-water insect, molluscs and crabs) and also fry (Larson and Murdy, 2001). Besides being native to tropical countries where the temperature remains mostly at 22°C - 28°C throughout the year (Riehl, et al., 1996), population of marble goby is also found in China and Taiwan (Randall, et al., 2000). Marble goby found in China is the Thai-native species which was introduced to the region in year 1988 (Larson and Murdy, 2001; Luong, et al., 2005). On the other hand, invasiveness of *Oxyeleotris marmorata* species from Cambodia into the ecosystem was conducted in year 1975 in Taiwan. However, regardless of the potential, for Fiji, more data and research on possible existence of *Oxyeleotris marmorata* needs to be collected and since there are none so far thus the population cannot be confirmed (Froese and Pauly, 2019). The effects of invasiveness or the introduction of non-native *Oxyeleotris marmorata* to the ecosystem are fairly unknown since there is a lack of research on the topic (Froese and Pauly, 2019).

A successful aquaculture needs adequate understanding of the fish being cultivated, its patterns and behaviours (Titin, et al., 2015) especially during its critical juvenile stages. “ikan hantu” is a common name refering to marble goby in Malaysia, and just as the name suggests, ‘ikan’ which translates to fish and ‘hantu’ which translates to ghost, describes the nocturnal and demersal behaviour (Riede, et al., 2004) of the fish which tend to hunt at night . The potamodromous (Riede, et al., 2004) *Oxyeleotris marmorata* is said to prefer depths of over 10 ft where they hide in rocks and vegetation (Froese and Pauly, 2019) and tend to half bury its body in the sand (Kelvin and Peter, 2002). This is done so in order to camouflage and ambush its prey. *Oxyeleotris marmorata* has a diet consisting of primarily of nekton at 60% as reported at Bukit Merah Reservoir, between September 1979 to August 1980 (Yap, 1988).

Oxyeleotris marmorata is significantly larger (Smith, 1945) compared to other members of goby family with maximum length reaching more than 50 cm in true length approximately to around 65 cm and weighing over 2 kg in total body weight (Senoo, et al., 1994; Kechik, 1995; Kottelat, 2001). The separated pelvic fin is what distinct marble goby from the other true-gobies (Bundit, 2007). *Oxyeleotris marmorata* has a darker dorsal side, light brownish vertical side and long dark blotches covers the body. The mouth has a profound split coordinated sideways verticle and the flattened upper-side of the head has ocelli (Kottlelat, et al., 1993). The fish has 7 dorsal spines with 9 soft ray, a single anal spine with 8 soft ray and has 60-65 pre-dorsal scales (Kottelat, 2001).

In the past few years marble goby has gained popularity and has become one of the most attractive candidate for aquaculture moving forward (Senoo, et al., 1994; Amornsakun, et al., 2003; Loung, et al., 2005). The gobloid fish is not only craved locally by the South-East Asian countries of Malaysia, Singapore, Thailand, and Indonesia (Roberts 1989; Senoo et al. 1993; Cheah et al. 1994; Amornsakun et al. 2002) but also in much of Eastern Asia (Rainboth, 1996). This attractiveness can be considered a result of the fish having white meat with fine texture and having taste to die for (Roberts 1989; Senoo et al. 1993; Cheah et al. 1994; Amornsakun et al. 2002) mouth-watering delicacies by means of frying, roasting or by simply steaming the fish can be prepared. The fish is also said to have no undesired off-taste or the unpleasant smell. Hence, demand for the marble goby is steadily rising and the supply is seen to be stagnant over the years due to shortage of seedstock (Seetapan, Puanglarp and Meunpol, 2012).

The *Oxyeleotris marmorata* is imported by Japan, Korea, China and even Singapore and Malaysia. *Oxyleotris marmorata* bids a hefty market price (Hoa and Yi, 2007; Wang et al., 2011) having its demand outstepping supply (Senoo, et al. 1993; Rakbankerd, 2005; Phoomthai, 2007). Indefinitely, this has made *Oxyeleotris marmorata* one of the most high-valued fish in the local and in the international market (Senoo, et al. 1993; Rainboth, 1996). The minimum retail price in Malaysia and Singapore of marble goby is 14 USD/ kg (Hoa and Yi, 2007; Wang, et al., 2011) and is said to rise up to RM86/ kg (Lam, et.al, 2008; Chew, et al., 2009; Loo, et al., 2013). Meanwhile, Amornsakun et al.

(2002) and Luong et al., (2005) reported a daily market price ranging from 18 to 20 USD/ kg.

Cultivation of marble goby is immensely crucial at present time as well as in the future. In Thailand marble goby is on top the list as a candidate for aquaculture (Suwanjarat, et al., 2005). The production of *Oxyeleotris marmorata* is merely just 0.1% of all the aquaculture production (FAO, 2016). A significantly low count considering the high demand. Obtaining data from all countries is rather difficult (Hoa and Yi, 2007; Wang, et al., 2011). However data from Malaysia, Singapore and Thailand for the year 2000 recorded around 207 tonnes marble goby were produced with 282 tons of gobloid fish produced worldwide (GALE, 2005). Leading the world in aquaculture production, Asia produced 85%, 89% and 92% of gobloid fish worldwidely in 1991, 2002 and 2004 respectively (FAO, 2004; FAO, 2006). Use of cages (Varangkana, 1986; Lin and Kaewpaitoon, 2000; Rakbankerd, 2005) and by the means of semi-intensive polyculture (Edwards and Allan, 2004) has been main contributor to the marble goby production. Much of the cultivated fish ends up in aquarium trade (Allen, 2011) and is considered crucial for consumers of Asian countries (Rakbankerd, 2005). Having both demand and a stable price throughout the year marble goby is appearing on both farmers (Cheah et al., 1994; Luong et al., 2005; Hoa and Yi, 2007) and researchers (Leatherland, et al., 1990; Jow, et al., 1999; Sayer, 2005; Masaya, et al., 2006) radar more frequently as the industry diversifies (Senoo, et al., 1994; Amornsakun, et al., 2003; Loung, et al., 2005).

The information on diseases concerning the marble goby is scarce and before 1978 there were next to none. During an experiment by the University Pertanian Malaysia (UPM) on the cage culture using *Oxyeleotris marmorata* encountered an unexpected high mortality rate. Further investigation found *Trichodina* sp. and *Henneguya shaharini* n. sp. Both part of protozoan parasite family to be the couse (Shariff, 1982). In Thailand, four parasite (protozoa, arthropods trematodes and fungi) infections were reported to infect marble gobies being cultivated in cages by Supamataya, (1984) at Nan River of the Nakornsawan province. Meanwhile, *Aeromonas hydrophila,* a bacterial infection was also prominent (Supamataya, 1984). In the case of virus, sand goby virus (SGV) causes distinct ulcers on the skin, spleen and kidney of the marble gobies (Hedrick, et al., 1986). Most of these diseases are directly caused by the poor quality of water (Lam, et al., 2008) and thus a proper re-circulation system needs to be deviced by which water can be filtered and recycled to provide clean environment for the fish while avoiding pollution. (Lam, et al., 2008).

2.3 Difficulties of *Oxyeleotris marmorata* Breeding, Cultivation and Its Feedstock

Oxyeleotris marmorata is a carnivorous freshwater fish with a high demand in the Southeast Asia usually fetching a high wholesale price (ranging from RM 80–160/ kg in Malaysia) credited to its delicate fillet texture (Darwis, et al., 2009), lean body and their firm, white flesh which has no off flavour taste

(Sompong, 1980). The aquaculture production depends greatly on the ability of successful rearing throughout the early life stages. (Darwis, et al., 2009). The breeding, cultivation and the feedstock are key aspects that needs urgent attention in order for large domestication of marble goby.

2.3.1 Tough Challanges in Breeding of *Oxyeleotris marmorata*

Cultivation of marble goby is considered toughest among the fish cultured in Malaysia due to the lack understanding and lack of enough research. Any fish regardless of species are at its most vulnerable during the juvenile period this is especially true for marble goby (Senoo, et al., 1994). Low survival rate, slow growth, bottom feeding habits and also the lack of specified feed has been hindering years of efforts to formulate an effective culture techniques for the pricy *Oxyeleotris marmorata* (Lam, et al., 2008; Chew, et al., 2009; Loo, et al., 2013). Even though there's numerous culture systems has been deployed and modified previously. However, there are still significant problems that needs attention (Lam et al., 2013).

Population density and shelter are the two aspects which has been the key to improving both survival and growth rate of some species at early life stages (Gwak, 2003; Gibtan, et al., 2008; Benhaïm, 2009). For non-carnivorous species such as Artic-char and black sea bass shelter helps protect the population from predators and the environment thus increasing the production diversely.

However for some species an opposite trend is observed where density has a negative impact and, so, providing shelter for such species becomes inane (Gibtan, et al., 2008)

The dwindling numbers of *Oxyeleotris marmorata* in the nature is a pressing concern. This reduction is a direct consequence of over-fishing (Darwis, et al., 2009) and is also due to the pollution of the rivers, lakes and ponds. The aquaculture population of the marble goby so far still solely relies on the seeds collected from the wild, thus these remaining population needs to be managed sustainably. Successes in breeding techniques in the hatcheries (Tavarutmaneegul and Lin, 1988) is nonetheless a breakthrough however the issues of mass production of an average of 50 g of the fingerlings (Tavarutmaneegul and Lin, 1988; Senoo, et al., 1994b) and also the turtle paces growth requires further research. In spite of the fact that the fish have been effectively produced by both natural (Phinal, 1980) and artificial spawning (Tan and Lam, 1973), there has been little to none effort put into fry rearing and little attempts at large production of stackable size fingerlings (Hoa and Yong, 2007). In order to attain stable technique for mass seed breading, the issue created by the low survival rate in the first 40 critical days (the juvenile stage) needs to be solved (Darwis, et al., 2009). Daily food intake, feeding activity and growth of *Oxyeleotris marmorata* juveniles reared under different salinity levels. Attempts at seed breeding of marble gobies in Singapore (Tan and Lam, 1973), Thailand (Tavarutmaneegul, et al., 1998), and Malaysia (Cheah, et al., 1991; Senoo, et al.,

1994) has been achieved but the suicidal starving posed by the juveniles prevents mass production since most of the hatchling is likely to reject feeding and die within the 41 day period (Senoo, et al., 1991). In a 12 months period the weight growth of the marble goby is only 45 g (Hoa and Yi, 2007) which is insignificant when compared side by side to other freshwater fishes. Mono-sex tilapia reach marketable size as early as 2 to 3 months of rearing weighing around 100 g to 150 g and the size varies depending of culture systems, feed and water quality (Begum, et al., 2017) meanwhile sea-beam takes 1.5 year to reach harvesting size and barramundi reaching size of 350 g to 3 kg in just 6 months to 2 years as reported by FAO Cultured Aquatic Species Information Programme (CASIP). It is agreed thus far that an effective nursing technique to source marble goby juveniles is the best solution to protect the remaining wild indigenous population of marble goby (Hoa and Yi, 2007).

However, the seed production of *Oxyeleotris marmorata* in hatcheries is still inconsistent and most of the seedstock are caught from the wild (Amornsakun, et al., 2003; Loo, et al., 2013). Experiments on egg and larval development were first carried out many years ago in 1991 (Senoo, et al., 1994). The brood fish were collected from earthen fish ponds in the State of Selangor, Malaysia and were reared in tanks for 8 months. Secondary identification of sexual characteristic is essential in broodstock management programme in order to develope viable and sustainable aquaculture production (Hasan, et al., 2012). One of the problems appears in mega scale production of marble goby highlighted by local farmer is difficulty to identify sexually mature broodstock

(Idris, et al., 2012).

2.3.2 *Oxyeleotris marmorata* Cultivation Culture and Its Environmental Factors

Use of cage culture in Thailand can be traced back to the early 1970's where fishes were being domesticated in pond (Suwansart, 1979). Department of Fisheries Malaysia (1981) recorded total cage production of 560 tonnes. Besides cages, ponds and cove have also been utilized for domestication of marble goby in Malaysia (Jee, 1980), Thailand (Cheah, et al., 1994; Menasveta, 2000), Vietnam (Luong, et al., 2005), and Cambodia (MAFF, 2005).

Polyculture utilizes the concept of niches to maximize productivity per unit area of the habitat. A properly planned policulture can considerably increase productivity by creating a stable ecosystem either by having an environment with no interspecies interaction with unlimited interactions with ecological, competiveness and predation. For example in order to increase the productivity of the marble goby, caged semi-intensive polyculture is deployed with

population of marble goby, giant fresh water prawn (Lin, et al., 2002), sea bass (Seenoo, et al., 1994) and tilapia (Sompong, 1980) together alongside with various captured based fisheries (Bundit, 2007) are raised together. In this way healthy and strong population of fish in the culture can be maintained, this is a big advantage of polyculture over monoculture.

Though cage culture has been a main contributor of the aquaculture industry, however the associated glitches particularly concerning reduced water quality (Lam et al., 2008) cannot be overlooked (Cheah, et al., 1994; Lin and Kaewpaitoon, 2000). Dirty and unsanitary water quality for marble goby triggers aggressive and cannibalistic nature of the fish due to stress and also exposing the fish to the unwanted and deadly diseases (Tavarutmaneegul and Lin, 1987) which greatly effects the productivity (Lam et.al, 2013). Thus, efforts are being poured into developing an effective and efficient re-circulation aquaculture system (RAS) in some part of the world where majority of the filtered clean water could be recycled back (Lam, et al., 2008) while the un-recycled water is safely channelled backed into the environment safely avoiding negative impact on the ecosystem and biodiversity at our own peril.

RAS truly has the potential that can be a better alternative for aquaculture providing controlled stress-free culture conditions especially the water quality (Senoo, et al., 1994; Lam, et al., 2013) which in return can lead to

higher survival rate among the marble goby stocks and may also improve growth rate (Tng, et al., 2008). It is unsure the results of using RAS on extensive and semi-intensive cultures due to insufficient information (Lam, et al., 2013), however for an intensive culture it is shown to diminish use of land by limiting the water discharge while guarding the environment (Lawson, et al., 1995; Davis, et al., 1998; Arbiv, et al., 1995) especially when RAS is used for fishes such as sea-bass (Franco-Nava, et al., 2004), African catfish (Endut, et al., 2010) and carp (Martins, et al., 2009).

To design an effective re-circulating aquaculture system for marble goby (Lam et.al, 2013), the amount of excretion of the indigestible feed content in form urea is required as ammonia is poisonous (Cooper and Plum, 1987; Butterworth, 2002; Felipo and Butterworth, 2002; Ip, et al., 2004; Chew et al., 2006).

Teleost often increase their ammonia excretion after eating. However, there would be a brief postprandial rise ammonia concentration in blood (Kaushik and Teles, 1985; Wicks and Randall, 2002), and the brain would have to protect against ammonia toxicity by increasing glutamine synthesis (Wicks and Randall, 2002; Ip, et al., 2004; Chew, et al., 2006). As reported by (Tng, et al., 2008), glutamate levels in the liver and gut of juvenile *Oxyeleotris marmorata* rise 6 hours after feeding. Considering the rate of glutamate catabolism in the liver being lower than the overall rate of glutamate release, this consequently, increases in hepatic glutamate dehydrogenase (GDH) amination activity and intestinal GDH activity in juvenile *Oxyeleotris*

marmorata and thus excreting 33% of ingested nitrogen during the 24 hours post-feeding (Tng, et al., 2008)

2.3.3 Challenges of *Oxyeleotris marmorata* Feedstock

Peculiar feeding behaviour in particular is one of the biggest torn in sight. The faster growth of associated with ability of the fish to consume and digest more feed (Silverstein, et al., 1998). Therefore, there is a need for a formulation feed specified for the juvenile marble goby (Lin and Kaewpaitoon, 2000) as feed is a factor that do hold an essential role in farming and mass production of marble goby (Darmawiyanti, 2005; Darwis, et al., 2009; Kordi and Ghufran, 2013; Tintin, et al., 2015). In the early nutritional development of feeds for capture-based carnivorous finfish species such as snakehead (Jantrarotai, et al., 1993) and grouper, refusal to accept feed was a prevalent concern (Kevin and Michael, 2005). There is also currently scarcity in the study on the use of farm-made aquafeed for marble goby, despite the fact that farm-made aquafeed have long been a key component of growing-out production (Edwards and Allan, 2004; Suchart, et al., 2005). Small-scale aquaculture producers in Asia and the Pacific have long recognized the advantages of utilizing farm-made aquafeed (Tacon, 1997; Tacon and Silva, 1997) and it is well established that production of finfish and crustacean fed with farm-made aquafeed rounds up to around 33% of the total production (Kee-Chai, 1993; Francesca, et al., 2004). According to FAO (1993), roughly 2.0 to 2.3 million metric tonnes of worldwide carnivorous finfish output and around 25 million metric tonnes of crustacean production

were generated without the use of commercial feed in 2004. The fact that farmers can tailor feed inputs to their own financial resources and needs using farm-made aqua feeds allows for cost saving by including locally available by-products of agriculture (Edwards, Le Anh Tuan and Allan, 2004).

Feed is the most expensive component in aquaculture, accounting for more than 30% to 60% of overall costs, depending more or less on the level of farm intensity (De Silva and Anderson, 1995). Aqua-feed is costly and the price has been spiking over these past few decades. Similar to any other food product, it has an expiration date, thus most of the feed available are general and not specified. Thus, usually specified feed for aquaculture is prepared by farmers themselves. Any good aquafeed usually contains fishmeal, fish oil, binder (corn-starch), vitamins and minerals. Fish oil and also fishmeal has become less readily to come-by nowadays, thus with it increasing market value has amplified the search for an alternative sources (Sprague, et al., 2016; Paulino, et al., 2018). The vegetable oil being abundant in nature is a promising candidate in substituting fish oil since it is cost-friendly, sustainable in the long run and also safe for the environment (Turchini, et al., 2009; Teoh and Ng, 2013). Fish oil is the main source of lipid in an aquafeed (Ti, et al., 2019) since carnivorous fish of all species have problems digesting carbohydrates, fatty acid a source of energy is essential in sustaining healthy growth especially for the fry (Pei, et al., 2004). So far, the results of substituting fish oil with the vegetable from various sources has not shown any unwanted side-effects on the growth rate of the fish and the requirement of the fatty acid was fulfilled (Senadheera, et al., 2015;

Senoo, et al., 1994; Thanuthong, et al., 2011).

Carnivorous fish are known to utilize protein as energy more efficiently than lipids and carbohydrates (Du, et al., 2005). Several studies reported that unsuitable dietary lipid levels can adversely affect the growth, body composition and health of fish (Tucker, et al., 1997). It may also negatively influence the ability of fish to digest and assimilate fatty acids (Ruyter, et al., 2000). However, several studies report that increasing dietary lipid within a certain limit can improve the growth and protein utilization of fish through the protein sparing effect by lipid (Peres, et al., 1999; López, et al., 2009; Kim, et al., 2010), where dietary lipid is used as the main energy source in the overall energy expenditure while dietary protein is utilized for growth (Xu, et al., 2001). However this protein sparing effect of high dietary lipid is missing for the marble goby. Thus lower lipid utilization is observed (Yong, et al., 2015). Bundit (2007) also suggested that poor growth and survival rate associated with artificial feed are caused by low digestibility for protein in *Oxyeleotris marmorata*. It has been reported that marble goby is a lean fish which more likely to utilise monounsaturated fatty acids (MUFAs) for energy source and could have the ability in bio converting linoleic acid (LA) to arachidonic acid (ARA) (Bundit, 2007).

In terms of fish meal, various efforts had been undertaken to find alternative protein sources to replace fishmeal in feed production (Francis, et al., 2001; Shapawi, et al., 2007; Hardy, 2010; Cheng, et al., 2010; Yu, et al., 2012).

Fish meal has been reported to have the highest cost and contributing to the high price of the aqua-feed. From year 2011 to the end of 2020 the price of the fish meal is vividly seen to have doubled in value. Fish meal in general is the source of protein in the feed which traditionally is the blending of fish bones and the left over from the wild fisheries which by trend has seen a drop where less and less fishes a being landed, thus there is a lack of the left over driving the price higher. As a solution, the use of plant based as an alternative has been widely explored. One such substitute has been the soybean meal receiving backlash from the anti-nutritional factor has a limited digestibility in monogastric animal such as fish (Bureau, et al., 1998; Peres, et al., 2003). Young marble goby juvenile can utilize 10% of defatted soybean meal in their diet without affecting its growth, nutrient utilization and intestinal condition (Yong, 2013). Compared to the most other carnivorous fish, marble goby has a lower tolerance , where other carnivorous fish has tolerance of up to 40% (Tantikitti, et al., 2005; Kikuchi and Furuta, 2009; Yigit, et al., 2010; Lim, et al., 2011; Yu, et al., 2012; Antolović, et al., 2012).

Artificial feed when compared to live-feed is less laborious, cheaper and safer (Hoa and Yi, 2007). In a previous study, it was found that juvenile marble goby can take fish meal-based pellets and that defatted soybean meal can substitute 10% of dietary fish meal without compromising development, nutritional utilization, or intestinal condition (Yong, et al., 2013). Later research by Yong et al. (2015) found that 12% dietary lipid seems to be optimal for juvenile marble goby development. Because there are no commercially

available feed for marble goby, many farmers are forced to use a variety of self-prepared random diets, including farm-made aqua-feed, trash fish, and commercial feeds for other species, which can lead to nutritional problems like excessive fat accumulation, fatty liver, and mortality (Bundit, 2007).

Despite the fact that many attempts have been made in recent years to effectively improve cultivation procedures for marble goby, yet the problem still persists (Lam et.al, 2013). This is due to its sluggish growth, high death rate, unusual eating behaviour, and absence of formulated feeds during the juvenile stage (Lam et al., 2013). In Malaysia (Yap, 1988), juvenile marble goby preyed mostly on nekton and finfish while in Thailand (Duangsawadi, et al., 1992) the fry and shellfish mainly prawn similar prey as in Malaysia was observed ingested by the wild population. Thus as expected, similarly marble goby raised in cove consumed freshwater prawn *(Macrobrachium sintangense)*, *Clupeichthys* sp. fry and also benthos in Tri An reservoir, Vietnam (Ti, et.al, 2019). When it comes to feed, zooplankton is the general or the benchmark feed in aquaculture (Hecht and Appelbaum, 1987). However, artificial feed and trash fish are also used widely (Le, 1996; Millamena and Toledo, 2004; Williams and Rimmer, 2005). For example, in the case of African sharp tooth (Hogendoorn, 1980) and humpback grouper (Sugama, et al., 2001), brine shrimp (*Arthemia*) was the best feed (Senoo, et al., 1992). The prefer-ability of feed of the fish differs from one fish to the other. Therefore, the feeding behaviour and digestive mechanism of the fish must first be established in order to formulate the best possible feeding procedures (Sua'rez, et al., 1995). Once the ontogenetic profile

from the digestive enzyme is obtained, prediction on the feed composition could be made to optimize the growth (Hofer and Ko'ck, 1989). In order for the growth (liver and intestine) 67% of nitrogen content is seen retained by the marble goby (Lim, et al., 2017).

As discussed earier, fish are at their most vulnerable early in the life during the juvenile stages where ingestible food is scarce. It is a fact that fish prey on fish only as big as their mouth (Kisalioglu and Gibson, 1976). Larval marble goby feed on artificial feed prepared using zooplankton (*Brachionus sp*., green water, *Moina sp*) had positive effect on the survival rate of the juveniles (Senoo, et al., 1993) especially the *Brachionus* sp. (Lin, 1988). Refusal to consume feed caused most of the stock juvenile to starve and die (Tan et.al, 1973) even when the pellet size was small enough for the juveniles to ingest (Senoo, et al., 1993). Development sense organ has an impact on the ontogenetic changes of behaviour (Kok, et al, 1980; Cheah, et al., 1994; Nhi, et al., 2010).

One of the factors contributing to the inconsistence in seed production of *Oxyeleotris marmorata* is related to their feeding at the grow-out stage. *Oxyeleotris marmorata* rejects formulated feeds (Rojtinnakorn, et al., 2012) and mass mortality usually occurs during a long deterring period due to starvation (Merican, 2011). The problem is mainly due to the poor palatability of the feeds which can be solved by supplementing and adding suitable feeding stimulant into the diets (Person-Le Ruyet, et al., 1983; Person-Le Ruyet, 1989; Kubitza and Lovshin, 1997; Kubitza, et al., 1999; Hirt-Chabbert, et al., 2012; Horváth

et al., 2013). Food stimulant is a taste substance that promotes a high ingestion rate in fish by stimulating the taste (Kasumyan and Døving, 2003). However, the effectiveness of these stimulants in *Oxyeleotris marmorata* feed is unknown. Multiple use of taste sedative is better compared to using just a single (Ohsugi, et al., 1978; Mackie, et al., 1980; Lim, et al., 2015). However, stimulants basically multiple amino acids, nucleotide and nucleoside are expensive, hence a more cost-saving substances that can promote taste for *Oxyeleotris marmorata* (Lim, et al., 2017) such as the use of organic acids, sugars and salts have seen to act similarly as amino acids, nucleotide and nucleoside (Kasumyan and Døving, 2003). The drop in pH has shown positive ingestion rate in *Oxyeleotris marmorata* suggesting that the fish prefers acidic food. Evidently, all the organic acids and aspartic acid tested were potential feeding stimulants for *Oxyeleotris marmorata* (Lim, et al, 2017).

It has been well known that salinity affects the daily food intake in fish. Thus, any disruption of osmoregulation may cause mass death among the stock of marble goby being cultivated (Senoo, et al., 2008). Thus the optimum salinity of the rearing water is 10 ppt to ensure greatest rate of survival (Darwis, et al., 2009). In *Oreochromis niloticus* (Nile tilapia), the energy needed for osmoregulation appears to influence feed consumption and development rate. If the fish were raised in an isosmotic environment, the energy needed for osmoregulation and basic metabolism would be reduced, resulting in more efficient food consumption and faster development. Hence, the salinity is considered in the experimental trial for the study of marble goby.

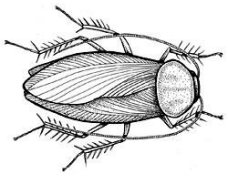
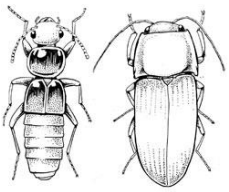
2.4 Insects Meal as Potential Feedstock

Human population has increased drastically and reached 8 billion in November 2022 (United Nations, 2022). Without a proper food supply chain, half of the population in the world will suffer from food hunger. In order to solve the food supply issue, an intensified food production system should be implemented (Crist, Mora and Engelman, 2017). However, animal based food supply such as cattle, poultry and fishes are facing the financial problem due to high raw material cost for the feed production. Thus, from the point of views of circular economy and sustainable development growth, the insect is the best choice to play the role as lipid and protein source for agriculture sector.

From the global bio-diversity, approximately 1 million of insect species can be found (Erwin, 2004). However, not all of them are suitable to use as animal feed and consumed by human. Approximately around 1,900 species of insect are consumed from different continents (van Huis, et al., 2013). These palatable insects are consumed in different form of larval stages such as eggs, larvae, pupae, nymph and adult. It can be easily obtained from the nature, semi-domestication from the wild and sustainable farming (Yen, 2015). According to the studies done by academicians and researchers, the insect species commonly used as animal feed ingredients and human food are generally from the orders such as Blattodea, Coleoptera, Diptera, Isoptera, Lepidoptera, Orthoptera

(Sanchez-Muros, Barroso and Haro, 2016). Table 2.1 shows the insect species used in accordance to its order categories.

Table 2.1: Summary of Insects Commonly Used as Animal Feed or Feed Ingredient

Insect Orders	Insect Species	Diet application	References
 **Blattodea** (Cockroaches)	American cockroaches (*Periplaneta americana L.*)	Fed to 4-week-old birds, *Gallus gallus domesticus* L	Aigbodion, Nathaniel Egbon and Erukakpomren (2012)
 **Coleoptera** (Beetles)	Oil palm weevil (*Rynchophoru phoenicis, Rynchophoru ferrugineus, Rynchophoru palmarum*)	A part of human diet in Kwara State, Nigeria.	Fasoranti and Ajiboye (1993)
	Rhinoceros beetle (*Oryctes spp.*)	A part of human diet in Kwara State, Nigeria.	Fasoranti and Ajiboye (1993)
	Superworm (*Zophobas morio.*)	Fed to Juvenile Nile tilapias (*Oreochromis niloticus*).	Jabir, Jabir and Vikineswary (2012)
	Lesser mealworm (*Alphitobius diaperinus* Linnaeus)	Remark: No specific application in paper.	Crippen, et al. (2012)
	Yellow mealworm (*Tenebrio molitor Linnaeus*)	Remark: human and animal consumption. Feed for broiler	Ramos-Elorduy, et al. (2002) Oonincx, et al.

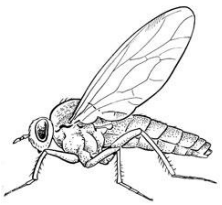
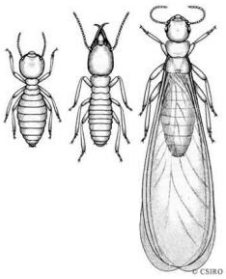
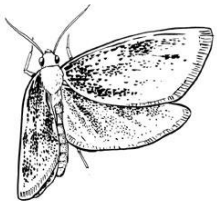
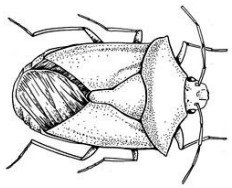
		chicken, saltwater fish (sea bass)	(2010) Gasco, et al. (2016)
 **Diptera** (Flies and Mosquitoes)	Common house mosquito (*Culex pipiens L.*)	Remark: Starvation induced cannibalism of Common house mosquito (*Culex pipiens L.)*	Husseiny, et al. (2018)
	Housefly (*M. domestica*)	Fed to Bullfrog, *Rana (Lithobates) catesbeiana*	Li, et al. (2019)

Table 2.1: (Continued)

	Black soldier fly (*Hermetia illucens*)	Fed to Fresh water Atlantic salmon *(Salmo salar)*	Belghit, et al. (2018)
	Blowfly (*Chrysomya megacephala*) maggot	Fed to farmed juvenile red tilapia (*Oreochromis sp.)*	Sing et al. (2014)
 **Isoptera** (Termites)	*Macrotermes spp.*	A part of human diet sub-Saharan Africa	van Huis (2003)
	Syntermes spp.	A part of human diet sub-Saharan Africa	van Huis (2003)
	Snouted termites (*Trinervitermes spp.*)	Remark (book): Notes On Ngangela And Nkoya Ethnozoology	Silow (1983)
 **Lepidoptera** (Butterflies and Moths)	Cutworm (*Agrotis infusa*)	Remark: The importance of moths is assessed in the subsistence economy of the region, and an analysis made of prehistoric food resources.	Flood (1980)
	Hawkmoth (*Daphnis spp.* and *Theretra spp.*)	Remark: Environmental manipulation	Van Itterbeeck and van Huis (2012)

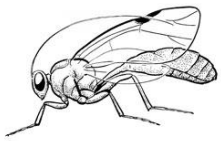
		for edible insect procurement.	
	Mopane caterpillar (*Imbrasia belina*)	Remark: farming for human consumtion.	Ghazoul (2006)
	Silkworm (*B. mori*)	N/A	Asimi, et al. (2017)
	Muga silkworm (*Antheraea assamensis* Helfer)	Fed to 630 day old straight-run Anak-2000 broiler chicks.	Sapcota, et al. (2003)

Table 2.1: (Continued)

 **Orthoptera** (Locusts, Grasshoppers and Crickets)	House crickets (*Acheta domesticus*)	Fed to 1-day-old, unsexed broiler chicks (Hubbard strain).	Nakagaki, Sunde and Defoliart (1987)
	Grasshopper (*Sphenarium*)	Remark: human food choices.	Cohen, Sanchez and Montiel-Ishino (2009)
	Mormon crickets (*Anabrus simplex* Haldeman)	Fed to I-day-old broiler chicks.	DeFoliart, Finke and Sunde (1982)
	Acrida cinerea (*Thunberg*)	Fed to 60-week-old Arbor Acres rooster	Wang, et al. (2007)
	Migratory Locust (*Locusta migratoria L.*)	Fed to 96 day-old unsexed broiler chickens (Abor acre)	Adeyemo, Longe, and Lawal (2008)
 **Hemiptera:** **Homoptera** (Cicadas, leafhoppers, planthoppers and	**Homoptera:** Cicada (*Ioba spp.*, *Platypleura spp.* and *Pycna spp.*)	Insect and other invertebrate foods of the Australian Aborigines.	Yen (2005)
	Cactus cochineal bug (*Dactylopius coccus*)	Insect and other invertebrate foods of the Australian Aborigines.	Yen (2005)

scale insects)			
Heteroptera (True bugs)	**Heteroptera:** Pentatomidae (*Agonoscelis versicolor*)	Remark : prospect of human comsuption - Edible insects: future prospects for food and feed security	van Huis, et al. (2013)

Table 2.1: (Continued)

	Aquatic hemiptera (*Corixidae spp.* and *Notonectidae spp.*)	Remark: Studies on the cephalic anatomy Naucoridae (Heteroptera	Parsons (2009)
 **Hymenoptera** (Wasps, Bees and Ants)	Weaver ants (*Oecophyll spp.*)	Remark: A historical review of research on the weaver ant Oecophylla in biological control	Van Mele (2007)
	Black weaver ants (*Polymachis dives*)	Remark : composition analyst of ant	Shen, et al. (2006)
	Yellow jacket wasps (*Vespula spp. and Dolichovespula spp.*)	Remark: The biodiversity of insects in Vientiane.	Nonaka, Sivilay and Boulidam (2008) Durst and Shono (2010)

In general, there is a lot of insects which can be used as animal feed. However, the eating habit and preferences of the animals must be considered when choosing the insect species. The species such as house fly maggots (*M. domestica*), blowfly (*Chrysomya megacephala*) maggot, grasshopper (*Sphenarium*), mealworm larvae (*Tenebrio molitor*), cricket (*Acheta*

domesticus) and waxworm (*Achroia grisella*) have been observed and evaluated through the feeding trials for certain species of animals but predominated by *Hermetia illucens* pre-pupae. (Wang, et al., 2004; Jabir, Jabir and Vikineswary, 2012; Finke, 2015; Gasco, et al., 2016; Hussein, et al., 2017).

2.4.1 Introduction of *Hermertia illucens* Larvae and Its Composition

The biochemical composition of the insect is very important in the study of the insect meal application as animal feed. However, the nutrient content of insect is mostly affected by its feed sources. The proximate analysis of *Hermetia illucens* is summarized in Table 2.2, with different feed sources in rearing process. In the study of Newton, et al. (1977) and Spranghers, et al. (2016) the *Hermetia illucens* larvae were reared on different organic waste such as beef cattle faeces and urine slurry, chicken feed, vegetable waste, biogas digestate and restaurant waste as shown in Table 2.2. Accoding to Newton, et al. (1977), Spranghers, et al. (2016), Liu, et al. (2017), Wong, et al. (2019) and Chia, et al. (2020) the crude lipid content was ranged from 21.8–38.6%, Crude protein content was ranged from 37.7–42.1% and 2.7–14.6% for ash content. However, the crude lipid content of the larvae fed on biogas digestate was 21.8% which was extremely low compared to larve fed on other feed sources. This can be explained by the carbohydrate content (energetic demand of *Hermetia illucens* larvae) of biogas digestate which was totally consumed by the microorganism

during methanogenic process (Spranghers, et al., 2016).

Table 2.2: Proximate Analysis of *Hermertia illucens* Larvae and Pre-pupae Fed with Different Organic Waste

Proximate analysis	*Hermetia illucens*							
Feed source	Beef cattle faeces and urine slurry	Chicken feed	Vegetable waste	Biogas digestate	Restaurant waste	Chicken Feed	Coconut endosperm waste	Spent barley
Larval stage	Larvae	Pre-pupae	Pre-pupae	Pre-pupae	Pre-pupae	Larvae	Pre-pupae	Larvae
Unit	% of dry basis	g/ kg	g/ kg	g/ kg	g/ kg	% of dry basis	% of dry basis	% of dry basis
Moisture (based on wet basis)*	NIL	613 ± 8	590 ± 10	614 ± 29	619 ± 10	NIL	NIL	NIL
Crude lipid (based on dry basis)	34.8	336	371	218	386	22.6 ± 0.15	25.88 ± 0.36	33.2 ± 1.24
Chitin (based on dry basis)	NIL	62	57	56	67	NIL	18.62 ± 1.25	NIL
Crude protein [a] (based on dry basis)	42.1 (Not chitin corrected)	388	377	401	407	38.0 ± 0.35 (Not chitin corrected)	37.70 ± 0.14	37.4 ± 0.62 (Not chitin corrected)
Crude ash (based on dry basis)	14.6	100	96	197	27	7.8 ± 0.41	NIL	8.3 ± 0.08
References	Newton, et al. (1977)	Spranghers, et al. (2016)				Liu, et al. (2017)	Wong, et al. (2019)	Chia, et al. (2020)

[a] The crude protein content obtained with chitin correction if not stated.

2.4.2 Application of *Hermertia illucens* Larvae as Aquatic Feedstock

The rapid development of agriculture sector leads to the shortage of traditional raw materials for husbandry and fishery animals. As the result, the material cost became very costly and increase the burden of the small-scaled farmers or businessmen. To turn over a new leaf, edible insect become the alternative material in animal feed for the industry. The nutritive compositions of insect studies showed the insect contained higher levels of protein and lipid in some species compared to animal meal (fishmeal) and plant meal (soybean meal) (Ramos-Elorduy, et al., 2002; Aigbodion, Egbon and Erukakpomren, 2012).

The insect meal used for the research studies were predominated by *Hermetia illucens* larvae and pupae which was widely studied for aquatic animal such as freshwater fishes and saltwater fishes (Lee, et al., 2012; Iaconisi, et al., 2017; Magalhaes, et al., 2017). According to Magalhaes, et al. (2017) *Hermetia illucens* pre-pupae meal can be used up to 19.5% of the diet which equivalently to 45% of fish meal in the diet without negative effects on the seabass growth performance. However, 13.0% *Hermetia illucens* diet showed better result in weight gained (162%), daily growth index was 2.28 and the feed efficiency rate was 0.62 in the study (Magalhaes, et al., 2017). Apart from seabass, a study conducted by Bruni, et al. (2018) used *Hermetia illucens* larvae meal instead of pre-pupae meal on rainbow trout (*Oncorhynchus mykiss*). The result showed highest growth performance in the rainbow trout fed with diet consisted 25% of

dietary fish meal replaced by *Hermetia illucens* larvae meal. The weight gained was 204.8% and the feed conversion rate was 0.88 after 78 days of feeding trial (Bruni, et al., 2018).

As mentioned above, the *Hermetia illucens* larvae and pre-pupae meal used were full-fatted. The studies conducted by Belghit, et al. (2018) and Dumas, et al. (2018) were more detailed on the effects of insect protein and insect lipid towards fish growth performances. The insect protein source was defatted *Hermetia illucens* larvae meal and the insect lipid extracted from dried fully fatted *Hermetia illucens* larvae through mechanical pressing process. The control diet in Belghit, et al. (2018) used fish meal and vegetable oil as the main protein and lipid source for the study of Atlantic salmon (*Salmo salar*). Based on the study, the diet with *Hermetia illucens* oil showed lower specific growth rate compared to the diet with vegetable oil. In spite of that, the protein source derived from *Hermetia illucens* larvae had no significant effects on growth performance (Belghit, et al., 2018). Dumas, et al. (2018) designed 7 diets included control diet, 3 defatted *Hermetia illucens* larvae meal diets with 25%, 50% and 100% of fishmeal replacement and 3 *Hermetia illucens* larvae oil diets with 25%, 50% and 100% of fish oil replacement. The growth performance of rainbow trout (*Oncorhynchus mykiss*) fed with diets contained defatted *Hermetia illucens* larvae meal was improved as the percentage of larvae increases. As report in Belghit, et al. (2018), Dumas, et al. (2018) had similar result in the larvae oil diets which no significant growth performance when the larvae oil level increase.

In addition, silkworm (*Bombyx mori L.*) pupae meal also popular in the study of insect meal. Based on the study of Lee, et al. (2012) diet with 10% of silkworm pupae meal replacement showed better growth performance on olive flounder (*Paralichthys olivaceus*, Temminck & Schlegel, 1846). The weight gained was 274.4%, specific growth rate was 3.13 ± 0.02 % per day, 1.12 ± 0.047 of feed efficiency ratio and 1.96 ± 0.082 of protein efficiency ratio (Lee, et al. 2012). However, the diet with 20% of 10% of silkworm pupae meal replacement showed lowest growth performance compared to control diet (without pupae meal). In the study of Jian carp juvenile (*Cyprinus carpio var. Jian*) by Ji, et al. (2013) dietary fishmeal protein was replaced with silkworm pupae meal at 0% (control), 50%, 60%, 70% and 80%. The result showed 50% pupae meal diet had the best growth performance among other pupae meal diets. However, the weight gained was 43.00 g slightly lower compared to control diet which was 45.33 g. Despite it, the feed conversion ratio (FCR) was surprisingly lower in 50% pupae meal diet (1.36 ± 0.08), whereas, the FCR for control diet was 1.41 ± 0.13. Hence, the silkworm pupae meal can be used to replace fish meal practically up to 50% for Jian carp for cost saving. Similar result obtained from Xu, et al. (2018) 50% of enzymatic hydrolysates defatted silkworm pupae meal on Mirror carp juvenile (*Cyprinus carpio var.* specularis) without any adverse effects.

The literature reviews of the use of *Hermetia illucens* larvae and pre-pupae or pupae meal as insect meal on specific fish species are summarized in Table 2.3.

Table 2.3: Application of *Hermetia illucens* Insect Meal in Freshwater and Saltwater Fish Species

Fish Species	Diet	CL	CP	Energy	References
European sea bass (*Dicentrarchus labrax L.*)	HM0	17.5	49.1	22.8 kJ g^{-1}	Magalhaes, et al. (2017)
	HM6.5	17.5	48.9	23.1	
	HM13	17.4	49.4	23.1	
	HM19.5	17.9	49.8	23.1	
Atlantic salmon (*Salmo salar*)	IM-0/VO (control)	18 %	47 %	21.7 MJ kg-1	Belghit, et al. (2018)
	IM-0/ IO1	19	46	21.8	
	IM-85/VO	22	44	23.2	
	IM-85/IO1	20	44	22.7	
rainbow trout (*Oncorhynchus mykiss*)	A (Control))	13.83 %	50.13 %		Dumas, et al. (2018)
	B 6.6M	14.38	49.03		
	C 13.2M	15.25	47.78		
	D 26.48M	16.52	46.92		
	E 2.5O	14.23	50.21		
	F 5.0O	14.60	51.12		
	G 10.0O	13.99	50.67		
rainbow trout (*Oncorhynchus mykiss*)	HI0	15.86	45.20 g/ 100g	About 22 MJ kg^{-1}	Bruni, et al. (2018)
	HI25	15.74	44.86		
	HI50	15.81	45.00		

2.5 Gaps of the Study

Based on the literature reviews, insect meal has widely used in the study for husbandry and aquaculture. However, no study uses insect meal for *Oxyeleotris marmorata* which widely found in Southeast Asia. *Oxyeleotris marmorata* is a carnivorous freshwater with high market pricing due to it delicate flesh texture and difficult to capture from river. In order to overcome the high consumption demand of *Oxyeleotris marmorata*, fish farming industry is putting efforts to exquisite and enhance the technical culture for *Oxyeleotris marmorata*.

Nevertheless, the problems of *Oxyeleotris marmorata* such as slow growth and high mortality rate are still unsolved.

The unusual eating habit of *Oxyeleotris marmorata* and lack of proper feed diet become the key points for the recent research study of *Oxyeleotris marmorata*. According to the study done by Lim, et al. (2017) used different organic acids as taste stimulant to enhance the ingestion of *Oxyeleotris marmorata* fingerlings. From the finding, *Oxyeleotris marmorata* is more favour towards aspartic acid and showed 94% of ingestion rate (Lim, et al. 2017). Other research studies, live tilapia fingerlings, live common carp fingerlings, minced scads rice field prawns were used as the feed based on the nature of *Oxyeleotris marmorata* to study its growth performance (Hoa and Yi, 2007; Lam, et al., 2008; Herawati, et al., 2016). Table 2.4 shows the research outputs from the literature reviews of *Oxyeleotris marmorata*.

Table 2.4: Research Studies Related to the *Oxyeleotris marmorata*

Diets/Feeds	Outputs of the study	References
Formulated fishmeal with different ratio of soybean meal	• The fishmeal can be replaced by defatted soybean meal up to 10% without adversely effects on the growth performance. • Growth performance, nutrients utilization has improved by the supplement of phytase.	Yong, Ooi and Shapawi (2013)

Table 2.4: (Continued)

Diets/Feeds	Outputs of the study	References
Agar gel pellet with amino acids, organic acids, sugars and classical taste substances.	• MG showed high ingestion rate (94%) towards aspartic acid. • Aspartic acid can acts as feeding stimulant solely compared to amino acid mixture.	Lim, et al. (2017)
Live Tilapia	• The feeding rate of 2% - 4% based on the MG body weight showed the increase of weight gained from 0.52 g/day to 0.82 g/day in second month. • Growth rate was decreased in third month (0.32 g/day to 0.44 g/day) compared to second month. • 4% of feeding rate was not the optimum feeding rate to maximize the growth performance.	Herawati, et al. (2016)
Live tilapia (*O. niloticus*) fry Live common carp (*Cyprinus carpio*) fry Minced scads (*Decapterus russelli*)	• MG fed with live tilapia fry had lesser waste excretion compared to the other two feeds. • Less waste excretion let to clearer cultured water which enhanced the survival rate and growth rate. • Diets such as live common carps fry and minced scads caused higher amount of nitrogen loss and poor nutrients utilization for MG.	Lam, et al. (2008)

Table 2.4: (Continued)

Diets/Feeds	Outputs of the study	References
Rice field prawn (*Macrobrachium lanchesteri*)	• The MG prefer smaller rice field prawn. • Small rice field prawns are easier to ingest by MG due to low prey speed. • Large rice prawn field with harder carapace and shell is not easy to digest by MG. • Ingestion period of MG peaked at night time. • Rice field prawn can be used as live food to train MG to accept artificial food.	Hoa and Yi (2007)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Overview of Research Study

Chapter 3 reports three phases of the research methodology carried out in this study. Prior to the experiments, the equipment, chemicals and materials such as *Hermetia illucens* (HI) larvae, experimental diets and *Oxyeleotris marmorata* (marble goby, MG) were prepared. In phase I, the farming of BSF pre-pupae using organic waste (fruit waste, consisted of jackfruit, papaya, banana, mango and guava) was conducted. The harvested larvae and pre-pupae was then processed into HI larvae and pre-pupae meals, and its proximate analysis were then carried out. Experimental diets with inclusion level 0%, 4%, 8% and 12% of HI meal were formulated to replace the fish meal. The proximate analysis of control diet and 3 different experimental diets was conducted to determine their nutritional content. In phase II of this study, a feeding trial using the formulated experimental diets were conducted on the MG to determine their growth performance in terms of specific growth rate, feed efficiency, feed conversion and survival rate. Phase III of the study determined the fillet yield and body composition of MG including fatty acids and viscerosomatic indices. The overall research study workflow is illustrated in Figure 3.1.

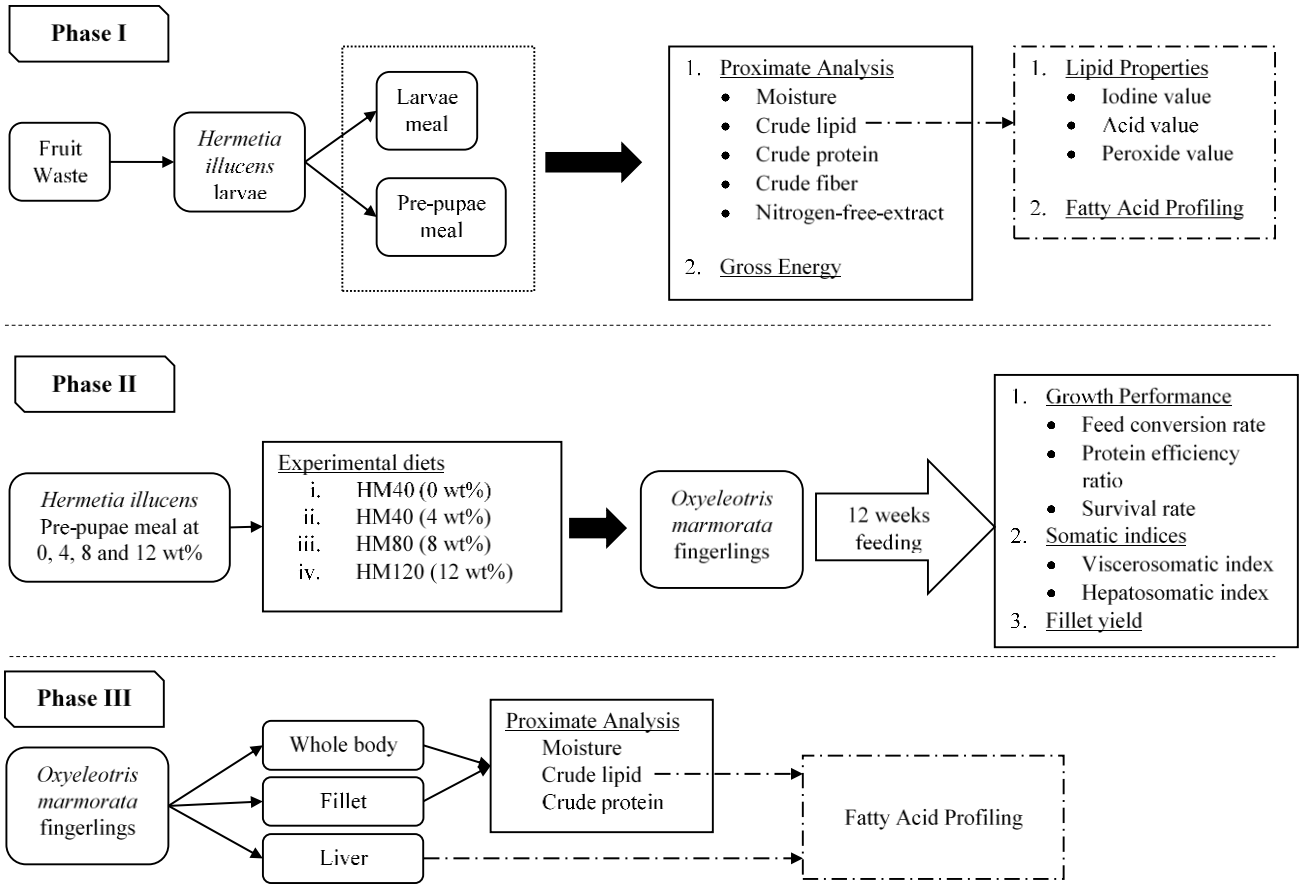


Figure 3.1: Overall Flowchart of Experimental Work

3.2 Farming of *Hermetia illucens* (Black Soldier Fly) Larvae

In order to farm *Hermetia illucens* larvae and pre-pupae, an enclosure was set up near the forest edge located at the University of Tunku Abdul Rahman Kampar campus. The colony of the *Hermetia illucens* larvae obtained was maintained from previous study done by Leong et al. (2016). The enclosure was self-designed which consisted of two tiers with four compartments as shown in Figure 3.2. The enclosure was fabricated from wood and lined with fine wire mesh, the dimensions were 90 cm in length, 70 cm in width and 90 cm in height. A pot of money plant, *pipremnum aureum* was put inside the enclosure for *Hermetia illucens* to mate. Water was sprayed twice at 7 am and evening 5 pm for *Hermetia illucens* to ingest and maintain the humid environment in the enclosure.

Figure 3.2: Front View of *Hermetia illucens* Breeding Enclosure

A tray of rotten fruits as shown in Figure 3.3 was put inside the enclosure for the female *Hermetia illucens* flies to lay eggs. Plenty of black filter balls were put together with the rotten fruits which provided more porous surface to enhance the *Hermetia illucens* egg laying. Figure 3.4 shows *Hermetia illucens* eggs were laid in the black filter ball. The *Hermetia illucens* eggs were collected every 3 days and a new tray of rotten fruits with black filter balls was placed.

Figure 3.3: Side View of the *Hermetia illucens* Breeding Enclosure

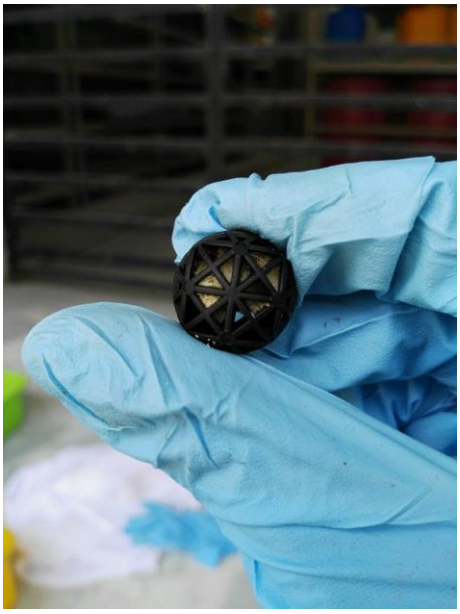


Figure 3.4: *Hermetia illucens* Eggs Harvested from the Filter Balls

The *Hermetia illucens* eggs were harvested from the filter balls which consisted of 4-5 lays of eggs and then were transferred to a new container (50 cm in length, 34 cm in width and 12 cm in height) for hatching and rearing. The container was wrapped with white cloth and tied up with rubber band to prevent the *Hermetia illucens* larvae from escaping. Hatching took about 3 days and the newly hatched *Hermetia illucens* neonates resided in the fruit waste as they eat and grow. After 3 to 5 days, neonates grew into approximately 0.5 – 1.0 cm larvae. The *Hermetia illucens* larvae were fed every day, the container was cleaned occasionally when it was full of unwanted pasty matters. Throughout the farming process, the larvae were fed with fruit waste such as (jackfruit, papaya, banana, mango and guava) collected from fruit retailers and distributors located at Kampar.

After 14 days of rearing, the *Hermetia illucens* larvae were harvested.

For *Hermetia illucens* pre-pupae harvesting, the rearing process continued further. After 18 days of rearing, *Hermetia illucens* larvae started to change colour and transformed into pre-pupae form. The *Hermetia illucens* pre-pupae climbed out from the container and were trapped inside the white cloth. The *Hermetia illucens* pre-pupae harvested were washed and oven dried at 80ºC for 24 hours until a constant weight was achieved. The dried *Hermetia illucens* biomass was stored in clean plastic bottles with food grade desiccant for proximate analysis and diet formulation.

3.3 Preparation of Experimental Diets

After the rearing of *Hermetia illucens* larvae, the pre-pupae with better quality in terms of chemical composition was selected and used as insect meal to partially or fully replacement for fish meal and fish oil in growth studies. The dried *Hermetia illucens* biomass were ground into powder form and is denoted as HI meal from hereon. It was used as alternative lipid and protein sources for the growth studies of *Oxyeleotris marmorata*.

3.3.1 Formulation of Experimental Diets

The experimental diets were made up of fish meal, fish oil, *Hermetia illucens* meal, wheat flour, corn starch, vitamins and minerals premix, carboxylmethyl

cellulose (CMC), alpha-cellulose and shrimp hydrolysate (Ti, Ong and Teoh 2019). The fish meal was substituted at 0% (HM 0), 4% (HM 40), 8% (HM80) and 12% (HM120) inclusion level by *Hermetia illucens* meal. These inclusion levels were also equivalent to 0%, 34%, 66% and 100% inclusion level of fish oil. The corn starch was used as carbohydrate source at 15% (weight/weight) of total ingredient. The vitamins premix and mineral premix made up of 3% and 2% of the formulated diet, respectively. The alpha-cellulose and CMC which functioned as filler and binder agent, were each used at 2% inclusion level. The shrimp hydrolysate at 1% inclusion level was used as feeding stimulant to boost feed uptake by the *Oxyeleotris marmorata* fingerlings (Ti, Ong and Teoh 2019). This formulation was designed and targeted to contain 9.5% crude lipid and 40% crude protein. The ingredients composition expressed in g/kg dry matter of the experimental diets (HM0, HM40, HM80 and HM120) are shown in Table 3.1.

Table 3.1: Ingredients Composition of Experimental Diets

	Diets			
	HM0	HM40	HM80	HM120
Feed Ingredients (g/ kg DM)				
Fish meal	590	567	543	520
Fish oil	50	33	17	0
Hermetia illucens meal	0	40	80	120
Wheat flour	110	110	110	110
Corn starch	150	150	150	150
Vitamin premix[*a]	30	30	30	30
Mineral premix[*b]	20	20	20	20
Shrimp hydrolysate	10	10	10	10
Carboxymethyl cellulose	20	20	20	20
Alpha-cellulose	20	20	20	20

[*a] Vitamin premix (content/ kg): Vitamin A, 50 MIU; Vitamin D_3, 10 MIU; Vitamin E, 130 g; Vitamin B_1, 10 g; Vitamin B_2, 25 g; Vitamin B_6, 16 g; Vitamin B_{12}, 100 mg; Biotin, 500 mg; Pantothetic acid, 56 g; Folic acid, 8 g; Niacin, 200 g; Anticake, 20 g; Antioxidant, 0.2 g; Vitamin K_3, 10 g.

[*b] Mineral premix (content/ kg): Copper, 7.5 g; Iron, 125 g; Manganese, 25 g; Zinc, 125 g; Cobalt, 0.5g; Iodine, 0.175 g; Selenium, 0.3 g; Anticake, 10 g.

The dry ingredients were weighed and placed into a mixer followed by wet ingredients. The mixture was mixed homogenously and minimum amount of distilled water was added to form a smooth dough using heavy-duty mixer (Figure 3.5). The dough was transferred into a screw-press extruder (Figure 3.6) the 2mm × 2 mm× 2mm of pelleted feed were formed. The pellets was oven dried in a convection oven at 50ºC for 24 hours, cooled and stored in freezer for further analysis and feeding trial.

Figure 3.5: Mixing of Dry and Wet Ingredients Using Heavy-duty Mixer

Figure 3.6: Screw-press Extruder Used for Fish Pellets Preparation

The proximate analysis of diets were carried out in accordance to AOAC Methods which were discussed in detail in section 3.4.

3.3.2 Feeding Trial of *Oxyeleotris marmorata*

The different ratio of HI meal substituted diets were used for the feeding trial of *Oxyeleotris marmorata*. The location of marble goby feeding trial was set at Aquaculture Facilities at Agriculture Park, Universiti Tunku Abdul Rahman with the coordinates 4.3426342, 101.1369382. The *Oxyeleotris marmorata* juveniles used were purchased from local fish shop, Weng Kee Akuarium which located at old town Kampar, Perak. Prior to the feeding trial, 240 *Oxyeleotris marmorata* fingerlings were allowed to acclimatize and trained with control diet. After 4 weeks of conditioning, only 120 strong and healthy *Oxyeleotris marmorata* juveniles were selected for subsequent feeding trial.

Thirty fingerlings were used for a control diet and three experimental diets. For each diet, triplicate of 10 *Oxyeleotris marmorata* juveniles were randomly distributed into an aquarium with the dimensions of 76 cm in length, 45 cm in width, 45 cm in height and 5 mm glass thickness as shown in Figure 3.7. Each aquarium filled with anti-chlorine treated tap water up to two-third of its 160 L capacity. In this feeding trial, recirculating water system was applied.

Figure 3.7: Aquarium Set Up for Feeding Trial of *Oxyeleotris marmorata*

In Figure 3.8 and Figure 3.9, the determination of *Oxyeleotris marmorata* length and weight are shown. The initial length and weight of the fingerlings were recorded at the beginning of the feeding trial. The feeding trial of *Oxyeleotris marmorata* juveniles were conducted for a period of 12 weeks and the juveniles were hand fed twice per day at 9 am and 5 pm until visual apparent satiation. The length and weight were determined once a week continuously throughout the feeding trial.

Figure 3.8: Determination of Length of *Oxyeleotris marmorata*

Figure 3.9: Determination of Weight of *Oxyeleotris marmorata*

3.4 Proximate Analysis

The proximate analyses carried out to evaluate the nutritional content such as moisture content, crude lipid, crude protein, crude fibre, ash content and nitrogen-free extract. The proximate analysis was carried out in accordance to AOAC standard methods and it was done in triplicates. Addition analysis, chitin

correction was carried out for *Hermetia illucens* meal to correct the true crude protein contributed by its biomass. The overall of proximate analysis chart is shown in Figure 3.10.

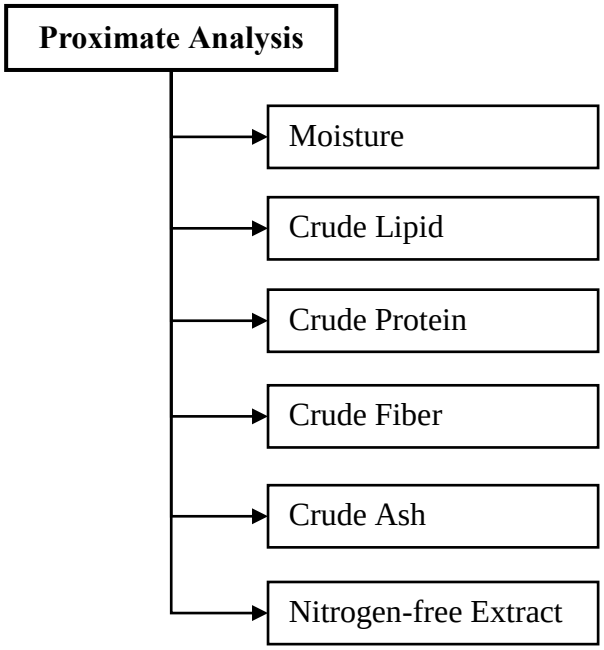


Figure 3.10: Determination of Proximate Analysis

The proximate analysis were carried out for the biomass of *Hermetia illucens* larvae, *Hermetia illucens* pre-pupae, control diet (HM0), experimental diets (HM40, HM80 and HM120), fillet and whole body of *Oxyeleotris Marmorata*.

3.4.1 Determination of Moisture Content

The moisture content of fresh *Hermetia illucens* larvae was determined using the method according to AOAC 934.01 described by AOAC (2012a). The

sample was subjected to oven drying (Memmert GmbH + Co.KG, Schwabach, Germany; Universal Oven UN110) to remove the moisture and then the remaining dry matters were weighed to determine the moisture loss.

The oven was preheated to 80 ºC before being used. The weight of an empty evaporating dish was measured, W_1. Approximately 10 g of the fresh sample was put evenly on the evaporating dish and weighted as W_2. The sample was dried in the oven at 80ºC for 24 hr. After the dried sample was removed from the oven, it was transferred immediately to the desiccator for cooling before being weight. The drying, cooling, and weighing process were repeated until a constant value was obtained. The weight of dried sample and evaporating dish were denoted as W_3. The moisture content in percentage was calculated based on the following equation:

$$\text{Moisture content, \%} = \frac{W_3 - W_2}{W_2 - W_1} \times 100\ \%$$

[Equation 3.1]

Where,

W_1 = Weight of empty evaporating dish, g

W_2 = Weight of fresh sample and evaporating dish, g

W_3 = Weight of dried sample and evaporating dish, g

3.4.2 Determination of Crude Lipid Content

Soxhlet method according to AOAC Method 920.39 was used to determine the crude lipid content of dried sample and described by AOAC (2012b). The extraction process involved continuous condensation of the heated organic solvent above the sample which builds up in the Soxhlet extractor chamber until it fills up. The organic solvent is drawn back into the flat bottom flask by the suction pressure created when the solvent exit from the siphon. The crude lipid content is determined measured by the weight difference.

Approximately 10 g of the dried sample denoted as S was weighed and ground using pestle and mortar. The sample was then put inside the thimble and placed in the Soxhlet extractor chamber. Before the Soxhlet extraction process, the weight of the empty flat bottom flask was measured and recorded as W_1. Approximately 150 mL of lipid solvent, n-hexane was measured and poured into the flat bottom flask. A heating mantle (Global Lab, republic of Korea; GLHMP-F25) was used to heat the n-hexane and allowed the sample to be refluxed for 8 hr at a rate of 10 refluxes per hour.

After 8 hours of reflux, the Soxhlet extractor together with the thimble were removed. The remaining n-hexane with dissolved lipid in the flat bottom flask was then double boiled using rotary evaporator (BÜCHI, Flawil 1, Switzerland; Rotavapor R-3) to remove residual n-hexane. The weight of the

flat bottom flask with crude lipid was weighed and recorded as W_2. The following formula was used to calculate the percentage of crude lipid extracted from the dried sample.

$$\text{Crude lipid content, \%} = \frac{W_2 - W_1}{S} \times 100\ \%$$

[Equation 3.2]

Where,

W_1 = Weight of empty flat bottom flask, g

W_2 = Weight of crude lipid extracted and flat bottom flask, g

S = Weight of dried sample used for crude lipid extraction, g

3.4.3 Determination of Crude Protein Content

The determination of crude protein content was carried out according to the AOAC Method 976.06 using the Kjeldahl method and described by Sáez-Plaza, et al., (2013) and Nielsen (2017). This method consists of 3 processes which are acid digestion, distillation and titration. The protein compounds and other organic components in the sample are digested by concentrated sulphuric acid with the presence of catalysts. During the acid digestion process, the total organic nitrogen converted into ammonium sulphate. The ammonium sulphate is neutralized with alkali which is sodium hydroxide and distilled into boric acid

solution using the distillation unit. The borate anions formed are collected and then titrate with standardized acid which is hydrochloric acid, HCl. The number of borate anions neutralized are equivalent to the number of nitrogen present in the sample.

Approximately 1 g of dried and defatted sample was weighed and recorded as S. 0.8 g of copper(II) sulphate, $CuSO_4$ and 7 g of potassium sulphate, K_2SO_4 were weighed and served as catalysts for acid digestion reaction. Twenty mL of 98% concentrated sulphuric acid, H_2SO_4 was measured. The sample, catalysts and acid were added into the digestion tube, accordingly. An empty digestion tube containing catalysts and acid only serve was used to as blank to correct nitrogen which might be present.

In the meantime, the Kjeldahl digester (BÜCHI, Flawil 1, Switzerland; speed digester K-436) was turned on and preheated to 410ºC while preparing the digestion tubes. The digester tubes with sample was inserted into the Kjeldahl digester. The suction module was pushed down straight onto the digester tubes. The scrubber (BÜCHI, Flawil 1, Switzerland; DuoScrub K-415) connected to suction module was switched on. Three litres of 20 % of sodium hydroxide, NaOH with bromothymol blue colour indicator was used in the scrubber to neutralize the acidic white fumes which escaped from the digestion unit. The digestion process took more than 4 hours for the sample to be fully digested. The digester tubes were then removed from the Kjeldahl digester and allowed to be cooled in the fume hood. While the digesta was still hot, 40 mL

of distilled waste was added drop by drop into the tube to prevent crystallization.

As for the distillation process, the semi-automated Kjeldahl distillation unit (BÜCHI, Flawil 1, Switzerland; distillation unit K-355) was switched on, the steam generator was preheated and the water tap connected to the distillation unit was opened. The two other tanks connected to distillation unit were filled with distilled water and 40 % of NaOH. Before the distillation of the sample, a digestion tube filled with 60 mL of distilled water was placed inside the distillator and an empty conical flask was placed at the receiving vessel compartment. The cleaning process was carried out for 2 minutes to ensure the distillation unit was clean and without other chemicals retained from previous use. Once the distillator is ready, a conical flask filled with 25 mL of 4 % of boric acid, H_3BO_3 containing the colour indicators cresolbromol green and methyl red was prepared and placed at the receiving vessel compartment to collect distillate. The receiving vessel was fully immersed in the receiving solution. The digester tube with digested sample containing 40 mL of distilled water was placed inside the distillator. 60 mL of NaOH from the tank was dispersed into the digestion tube and then the sample was allowed to distil for 3 minutes. The colour of the boric acid with indicators changed from pink to blue when the distillate was collected in the conical flask.

A titration unit was set up. 0.1 N of standard HCl was filled into a 50 mL burette and the initial volume was recorded as V_1. The volume of the HCl was used to titrate the distillate until the first colour changed from blue to pink

was recorded as V_2. The percentage of the nitrogen in the sample was calculated based on the following formula.

Percentage of nitrogen, %

$$\text{Percentage of nitrogen, \%} = N \times \frac{V_2 - V_1}{S} \times MV \times 100\ \%$$

[Equation 3.3]

Where,

N = Normality of HCl used for titration, mol/ 1000 mL

V_1 = Initial volume of HCl before titration, mL

V_2 = Final volume of HCl after titration, mL

S = Weight of sample used, g

MV = Molecular weight of nitrogen, 14.0067 g/ mol

The crude protein content can be calculated in the following formula based on percentage of nitrogen in the sample and factor conversion of 6.25.

$$\text{Percentage of protein, \%} = \text{Percentage of nitrogen, \%} \times 6.25$$

[Equation 3.4]

3.4.3.1 Chitin Correction

The procedure for chitin correction was carried out to eliminate the protein content of indigestible chitin from the exoskeleton of *Hermetia illucens* pre-pupae. The extraction of chitin was conducted by using two procedures which are deproteinisation and demineralisation (Puvvada, Vankayalapati and Sukhavasi, 2012; Chitra and Annadurai, 2012; Ali, 2013). Ten g of freshly ground sample was added into a 250 mL blue cap Schott bottle. An hundred fifty mL of 4% sodium hydroxide, NaOH was measured by using measuring cylinder and added into the Schott bottle. The Schott bottle was capped and placed into the orbital shaker for 24 hours at room temperature with 200 revolutions per minute. After 24 hours, the mixture was filtered by using filter paper, filter funnel, suction flask together with suction pump. The sample was washed with distilled water until the pH value dropped to neutral. This process is called deproteinisation. The demineralisation is the process to remove mineralized matter from deproteinised sample by using acid solution. The process is similar to deproteinisation except 4% NaOH was replced with 4% HCL. The process of both deproteinisation and demineralisation were repeated until the chitin extracted was clean with no residue left. The weight of chitin extracted was weighed and recorded. The yield of the chitin extraction as shown in Equation 3.5.

$$\text{Yield of chitin, \%} = \frac{\text{weight of chitin (g)}}{\text{weight of fresh sample}} \times 100\ \%$$

[Equation 3.5]

The protein content of the chitin extracted was determined by using the crude protein determination procedure.

3.4.4 Determination of Crude Fibre Content

Crude fibre content was determined using the AOAC Method 991.43. The method involves acid and alkaline digestion, filtered, over-dried followed by ignition of the digested sample. The weight difference of digested sample and ash collected after ignition was used to determine the crude fibre content. The reaction was conducted using the crude fibre analyser (Gerhardt, Germany, Gerhardt Fibrebag-Systems 6FBS) as shown in Figure 3.11.

Approximately 1 g of defatted sample was weighted and labelled as W_s. The sample was transferred into a fibre bag and a glass spacer was inserted. Subsequently, it was put inside a supported ring and fixed in a 1000 mL boiling beaker. For the acid digestion, 800 mL of 0.256 N sulphuric acid was added into the boiling beaker and preheated. A few drops of n-octanol was added to prevent foaming. The round bottom condenser with inlet and outlet was attached firmly on top of the boiling beaker. The heat was adjusted and boiled for 30 minutes. After the acid digestion, the fibre bag was removed and rinsed with distilled water until it became neutral in pH. After this, the supported ring with fibre bag was put back into the boiling beaker for subsequent alkaline digestion.

Eight hundred mL of 0.223 N of potassium hydroxide was added into the boiling beaker. A few drops of n-octanol was then added. The heat was adjusted and boiled for 30 minutes. After the alkali digestion, the fibre bag was removed and rinsed with distilled water until it becomes neutral in pH.

Figure 3.11: Crude Fibre Analyser (Gerhardt Fibrebag-systems 6FBS)

The fibre bag with digested sample was oven-dried at 105ºC for 24 hours. Afterwards, it was transferred into a crucible and put inside muffle furnace (LabTech, Republic of Korea; electronic muffle furnace, LEF-115S) at 600ºC for 4 hours for ignition. After 4 hours, the crucible was taken out from the muffle furnace and cooled in a desiccator. The weight of ash was weighed and denoted as W_1. A blank was conducted using a fibre bag without sample, the ash obtained was denoted as W_2. To calculate the percentage of crude fibre content, the following formula was used.

$$\text{Crude fibre content, \%} = \frac{W_1 - W_2}{W_s} \times 100\ \%$$

[Equation 3.6]

Where,

W_1 = Weight of defatted sample after ashing, g

W_2 = Weight of blank after ashing, g

W_s = Weight of defatted sample, g

3.4.5 Determination of Ash Content

The ash content was determined in accordance to AOAC Method 900.02 where the inorganic residues remaining after the removal of moisture and organic matters by heating at high temperature.

The weight of an empty crucible was measured, W_1. Approximate 1 g of dried sample was used and placed inside the crucible, the weight was measured and recorded as W_2. The crucible underwent ashing process at 550 ºC for 16 hr in the muffle furnace (LabTech, Republic of Korea; electronic muffle furnace, LEF-115S). After ashing, the crucible was taken out from the muffle furnace and cooled in the desiccator weighed, W_3. To calculate the percentage of ash

content, the following formula was used.

$$\text{Ash content, \%} = \frac{W_3 - W_2}{W_2 - W_1} \times 100\ \%$$

[Equation 3.7]

Where,

W_1 = Weight of empty crucible, g

W_2 = Weight of dried and defatted sample, and crucible, g

W_3 = Weight of sample and crucible after ashing, g

3.4.6 Nitrogen-free Extract (NFE)

Nitrogen-free extract is a calculation to represent the content of carbohydrates, starches and sugars. It was estimated by calculating the difference of dry matter to crude lipid content, crude protein content, crude fibre content and ash content. The formula to calculate the nitrogen-free extract content as shown below.

$$\text{NFE, \%} = 100 - \text{CL} - \text{CP} - \text{CF} - \text{Ash}$$

[Equation 3.8]

Where,

NFE = Percentage of nitrogen-free extract, %

CL = Percentage of crude lipid content, %

CP = Percentage of crude protein content, %

CF = Percentage of crude fibre content, %

Ash = Percentage of ash content, %

3.4.7 Determination of Gross Energy

Gross energy is defined as the total chemical energy of a sample measured in a bomb calorimeter, i.e. the heat energy released from complete combustion (Smit, Schönfeldt and Beer, 2004; Hall, Melendez and Jewell, 2013). Determination of gross energy is very important for nutritional facts.

The water bath, cooler, oxygen gas regulator, computer and bomb calorimeter (IKA, D-79219 Staufen, Germany; IKA calorimeters C 200) were switched on an hour before the sample measuring. The CalWin software was used for the measurement. Approximately 1 g of the dry sample was weighed and put inside the sampling holder. For the liquid sample, capsule was used. The sampling holder with thread attached was put inside the ignition adaptor and filled with pure oxygen gas. Cold water with the temperature ranging from 14 ºC to 16 ºC was used to fill the bomb calorimeter. The mass of sample was keyed

into the program and the measurement was ran automatically. After 17 minutes, the gross energy was shown on the display.

3.5 Lipid Properties

The properties of the oils extracted from *Hermetia illucens* larvae and pre-pupae were determined by peroxide value test, iodine value test and acid value.

3.5.1 Peroxide Value Test

The AOCS Method Cd 8b-90 was used to determine the peroxide value of the lipid. One g of lipid sample was weighed into a 250 mL glass conical flask. 30 mL of acetic acid–chloroform solution with the ratio of three to two was prepared using 50 mL measuring cylinder and poured into the conical flask. The flask was swirled gently until the lipid sample was fully dissolved in the solvent. 0.5 mL of saturated potassium iodide solution was measured by using 1 mL Mohr pipette and pipetted into the conical flask. The conical flask was stoppered and the contents were swirled for exactly one minute. Thirty mL of distilled water was added immediately by using 50 mL measuring cylinder into the conical flask. The flask was stoppered and shook vigorously to liberate the iodine from chloroform layer. A burette was prepared and filled with 0.1 N of sodium thiosulphate solution. The initial reading of the burette was recorded

and denoted as initial volume. The contents in conical flask was titrated slowly with well mixing until the colour lightens. One mL of starch solution was used as colour indicator and the colour of the contents in conical flask changed to blue grey colour. The contents was titrated continuously until the blue grey colour disappeared in the upper layer of aqueous solution. The final reading of the burette was recorded and denoted as final volume. The equation used to calculate peroxide value is shown in Equation 3.9. A blank sample was conducted as control.

$$\text{Peroxide value} = \frac{(S - B) \times \text{Normality of sodium thiosulphate} \times 1000}{\text{weight of sample (g)}}$$

[Equation 3.9]

Whereas,

S = volume of titrant used for sample, mL

B = volume of titrant used for blank, mL

3.5.2 Iodine Value Test

The AOCS Method Cd 1-25 was used to determine the iodine value of lipid. One g of lipid sample was weighed into a 500 mL iodine flask. 20 mL of chloroform was measured and poured into the iodine flask. The mixture was swirled and the sample was dissolved completely in the solvent. Twenty-five mL of Wijs solution was measured and pipetted into the flask. The flask was

stoppered, swirled, and stored in the dark for reaction to take place. After 30 minutes, the iodine flask was removed from the dark. 20 mL of 10% potassium iodide was added followed by 100 mL of distilled water. A burette was prepared and filled with 0.1 N of sodium thiosulphate solution. The initial reading of the burette was recorded and denoted as initial volume. The contents in iodine flask was titrated slowly with well mixing until the yellow colour almost disappeared. Afterwards, 2 mL of starch indicator was added. The solution changed colour from pale yellow to blue grey. Then, the titration was continued until the blue grey colour disappeared. The final reading of the burette was recorded and denoted as final volume. The equation used to calculate iodine value is shown in Equation 3.10. A blank sample was conducted as control.

$$\text{iodine value} = \frac{(B - S) \times \text{Normality of sodium thiosulphate} \times 12.69}{\text{weight of sample (g)}}$$

[Equation 3.10]

Whereas,

B = Volume of titrant used for blank, mL

S = Volume of titrant used for sample, mL

3.5.3 Acid Value Test

For the determination of acid value, the international standard method AOCS Method Cd 3d-63 was used. A 1:1 % of propanol and toluene was prepared. One

g of lipid sample was weighed into a 250 mL conical flask. Twelve mL of solvent mixture was added into the flask and swirled gently until the lipid sample was dissolved completely. Five to seven drops of phenolphthalein colour indicator was added into the flask. A burette was prepared and filled with 0.1 N of potassium hydroxide solution. The initial reading of the burette was recorded and denoted as initial volume. The contents in conical flask was titrated slowly with well mixing until the first permanent pink colour appeared and persisted for 30 seconds. The final reading of the burette was recorded and denoted as final volume. The equation used to calculate acid value is shown in Equation 3.11. A blank sample was conducted.

$$\text{Iodine value} = \frac{(S - B) \times \text{Normality of potassium hydroxide} \times 56.11}{\text{weight of sample (g)}}$$

[Equation 3.11]

Whereas,

S = volume of titrant used for sample, mL

B = volume of titrant used for blank, mL

3.6 Fatty Acid Profiling

Fatty acid methyl esters (FAME) was produced via in-situ transesterification.

Ten g of *Hermetia illucens* pre-pupae meal was weighed and ground. The methanol to sample weight ratio was fixed at 8.3 to 1. Fifty-two point four five mL of methanol was measured by using 100 mL measuring cylinder and added into the Schott bottle. The concentrated sulphuric acid was used as catalyst and the catalyst loading was fixed at 15.1 volume to volume percent in methanol. 7.92 mL of concentrated sulphuric acid was measured using 10 mL graduated cylinder and added into the Schott bottle. Ten g of the ground sample was added into the Schott bottle followed by 30 mL of n-hexane. The Schott bottle was capped and placed in the ultrasonic water bath which was set at 50ºC for 253 minutes. The temperature of the ultrasonic water bath was monitored throughout the reaction time.

After 253 minutes, the capped Schott bottle was removed from the ultrasonic water bath and was cooled to room temperature. Three different layers of aqueous solution were formed. The upper layer of FAME containing aqueous solution was pipetted out into a separating funnel. The aqueous solution was washed with 70º C distilled water to remove the impurity. The washed water was discarded. The washing was repeated several times until the washed water became clear. The top yellow oily layer was collected in a beaker and added with sodium anhydrous to remove the residual water content in FAME. The liquid sample was then poured carefully into a sample bottle with sodium anhydrous remained in the beaker. The sample bottle was then immersed in a 500 mL beaker containing 60 to 70°C distilled water and double boiling was carried out until the volume remained unchanged. The weight of the FAME was

weighed and recorded. The yield of the FAME was calculated using Equation 3.12.

$$\text{Yielding of FAME} = \frac{\text{weight of FAME (g)}}{\text{weight of sample (g)} \times \text{crude lipid content (\%)}} \times 100\%$$

[Equation 3.12]

The composition of FAME present in the samples were identified using Gas Chromatography – Flame Ionization Detector (GC – FID). The GC was equipped with a polar capillary column BPX 70 with dimension of 60 m × 0.25 mm × 0.25 μm. In this analysis, nitrogen gas was used as carrier gas with a flow rate of 4 cm^2/ min. The temperature of the oven was initially set at 155 ºC. It was then adjusted to 220 ºC with a rate of 4 ºC/ min and was hold for 10 minutes. The injection temperature was set at 250 ºC and the split ratio was 80 %.

The Supelco 37 component FAME mix with different concentrations (100%, 50% and 25%) were used as standard reference. The results obtained from these 3 concentrations were used to plot the standard curves. The equations were interpreted for further calculation.

The sample was first diluted with dilution factor of 50 (total diluted volume/ FAME volume) where 20 μL of FAME was mixed with 980 μL of n-hexane. One μL of sample was injected into the instrument with the split ratio

of 80: 1. The percentage of FAME composition (% mol/ mol) was calculated based on the peak area compared with standard curves.

3.7 Growth Performance of *Oxyeleotris marmorata*

The growth performance of *Oxyeleotris marmorata* fed control diet and experimental diets was determined by specific growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR) and survival rate. The length and weight of *Oxyeleotris marmorata* juveniles were measured every week throughout the feeding trial period. The feed consumption was recorded daily for each of the diets. The formulae for each of the growth parameters are as follows:

Specific growth rate based on weight gained (SGR_W), % growth/ day,

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\ \%$$

[Equation 3.13]

Whereas,

W_t = Weight of fish after feeding trial (g)

W_0 = Weight of fish before feeding trial (g)

t = Period of experimental feeding trial (day)

Feed conversion rate (FCR),

$$\text{FCR} = \frac{\text{Total feed consumed (g)}}{\text{Weight of fish gained (g)}}$$

[Equation 3.14]

Protein efficiency ratio (PER),

$$\text{PER} = \frac{\text{weight of fish gained (g)}}{\text{protein intake (g)}}$$

[Equation 3.15]

Condition factor,

$$\text{Condition factor} = \frac{\text{final weight}}{(\text{total length})^3} \times 100\ \%$$

[Equation 3.16]

Survival rate,

$$\text{Survival rate} = \frac{\text{final number of fish}}{\text{Initial number of fish}} \times 100\ \%$$

[Equation 3.17]

3.8 Determination of Biochemical Properties of *Oxyeleotris marmorata* After Feeding Trial

The quality of *Oxyeleotris marmorata* was evaluated after the feeding trial. Juveniles were first euthanized and dissected for their viscerosomatic indices and fillet yield determination. Fatty acid composition was analysed for the whole body, liver and fillet samples.

3.8.1 Somatic Indices and Fillet Yield

At the end of the feeding trial, *Oxyeleotris marmorata* fingerlings from each of the aquariums was randomly sampled, anaesthetised with tricane methane sulphonate (MS 222) and culled. The sampled *Oxyeleotris marmorata* fingerlings were dissected, skinned and filleted, the fillet was weighed for fillet yield. The viscera and liver were excised and weighed for the determination of viscerosomatic index (VSI), and hepatosomatic index (HSI) (Nunes, et al., 2011; Montenegro and Gonzalez, 2012; Zhang, et al., 2016). The formulae used as follow:

Fillet yield,

$$\text{Fillet yield, \%} = \frac{\text{weight of fillet (g)}}{\text{weight of whole fish (g)}} \times 100\ \%$$

[Equation 3.18]

Viscerosomatic index (VSI),

$$\text{VSI, \%} = \frac{\text{weight of viscera (g)}}{\text{weight of whole fish (g)}} \times 100\ \%$$

[Equation 3.19]

Hepatosomatic index (HSI),

$$\text{HSI, \%} = \frac{\text{weight of liver (g)}}{\text{weight of whole fish (g)}} \times 100\ \%$$

[Equation 3.20]

3.9 Statistical Analysis

All data collected from proximate analysis, lipid properties, growth performance and biochemical properties were expressed as mean and standard error mean (SEM) and used to determine the efficacy of the alternative lipid and protein source on the growth of the *Oxyeleotris marmorata* fingerlings. SEM was used as a guide to determine the precision of the estimated mean as well as the standard deviation. Standard length, total length and weight of the fingerlings were analyzed using Levene's test ($P>0.05$) to ensure homogeneity of variance between the fingerlings at the start of the growth study. Data were analyzed using one way analysis of variance (ANOVA) and treatment means were evaluated using Duncan's multiple range tests (to provide additional information on the differences between means) through SPSS Statistics 11.5 package (IBM, USA) where the significance was tested at 95% confidence level and significant differences were considered when P was less than 0.05.

CHAPTER 4

RESULTS

4.1 *Hermetia illucens* Larvae and Pre-pupae Meals

The characterizations of physicochemical properties are important for experimental diets formulation. The physicochemical properties including proximate analysis moisture content, crude lipid, crude protein, crude fibre and nitrogen-free extract were determined for laboratory prepared *Hermetia illucens* larvae meal and pre-pupae meal, and were compared with commercial fish meal. The lipid samples extracted from *Hermetia illucens* larvae meal and pre-pupae meal were also tested for their acidity, degree of unsaturation and degree of oxidation through determination of acid values, iodine value and peroxide values. Moreover, the fatty acid (FA) composition of oil samples from *Hermetia illucens* larvae and pre-pupae were analysed and compared with those of commercial fish oil. All the chemical analyses were performed in triplicate.

4.1.1 Proximate Composition of *Hermetia illucens* Larvae and Pre-pupae Meals

Table 4.1 shows the proximate composition of *Hermetia illucens* larvae, pre-pupae meals and commercial fish meal. The moisture content (wet basis) of *H. illucens* larvae meal and pre-pupae meal were 63.53 ± 0.04% and 62.69 ± 0.27%, respectively. However, the moisture content of fish meal obtained was 1.52 ± 0.02% which was based on dry basis. The experimental diets which was produced from oven-dried *Hermetia illucens* pre-pupae, contained about 1.52 ± 0.02% of moisture. The crude lipids in *Hermetia illucens* larvae and pre-pupae meals were significantly higher with the value of 47.39 ± 1.85% and 46.83 ± 1.37%, respectively. These amounts were three times higher than that in fish meal with the value of 15.59 ± 0.10%. On the contrary, the crude protein was significantly higher in fish meal (62.75 ± 0.27%) compared to *Hermetia illucens* larvae meal (39.77 ± 1.52%) and pre-pupae meal (43.84 ± 1.37%). Chitin, which was not present in fishmeal, was detected at 2.80 ± 0.23% and 6.09 ± 1.18%, respectively from the exoskeleton of *Hermetia illucens* larvae meal and pre-pupae meals. Additionally, the crude fibre, crude ash and nitrogen-free extract were found in vary amounts in all meals, with the values ranging between 1.31 – 3.68%, 5.33 – 18.08% and 0.32 – 3.65%, respectively. Moreover, the gross energy of *Hermetia illucens* larvae meal and pre-pupae meal were found in higher values, 27.20 ± 0.45 MJ/kg and 27.83 ± 0.11 MJ/kg, respectively, as compared to fish meal (20.33 ± 0.04 MJ/kg).

Table 4.1: Proximate Composition of *Hermetia illucens* Larvae and Pre-pupae Meals and Fish Meal

Proximate Analysis (% of dry matter)	HI larvae meal	HI pre-pupae meal	Fish meal	p-value
Moisture (% of wet basis)	63.53 ± 0.04	62.69 ± 0.27	1.52 ± 0.02[a]	<0.001
Crude lipid	47.39 ± 1.85	46.83 ± 1.37	15.59 ± 0.10	<0.001
Chitin	2.80 ± 0.23	6.09 ± 1.18	nd	<0.001
Crude protein	39.77 ± 1.52[b]	43.84 ± 1.08[b]	62.75 ± 0.27	<0.001
Crude fibre	1.31 ± 0.17	3.68 ± 0.30	2.15 ± 0.14	0.006
Crude ash	7.88 ± 0.44	5.33 ± 0.40	18.08 ± 0.01	<0.001
Nitrogen-free extract, NFE	3.65 ± 0.35	0.32 ± 0.53	1.43 ± 0.37	0.007
Gross energy (MJ/ kg)	27.20 ± 0.45	27.83 ± 0.11	20.33 ± 0.04	<0.001

Note: The data were stated in mean ± standard deviation in triplicate (n=3).
[a] The moisture content of fish meal based on dry basis.
[b] The crude protein value with chitin correction.

4.1.2 Properties of Oil Extracted from *Hermetia illucens* Larvae and Pre-pupae Meals

The AV and IV had the similar inclination all the way through larvae to pre-pupae. Surprisingly, PV were quite similar in larvae meal and pre-pupae meal, with 1.14 ± 0.24 mEq/kg and 1.11 ± 0.21 mEq/kg, respectively as shown in Table 4.2

Table 4.2: Properties of Oils Extracted from *Hermetia illucens* Larvae and Pre-pupae

Properties	Larvae meal	Pre-pupae meal	p-value
Acid value, AV (mgKOH/g)	2.01 ± 0.19	2.59 ± 0.58	0.125
Iodine value, IV (gI_2/100 g)	16.94 ± 4.37	23.76 ± 0.34	0.093
Peroxide value, PV (mEq/kg)	1.14 ± 0.24	1.11 ± 0.21	0.892

Note: The data were stated in mean ± standard deviation in triplicate (n=3).

In general, *Hermetia illucens* larvae contained approximately 80.7% saturated fatty acids (SFA), 14% monounsaturated fatty acids (MUFA), and 6.2% polyunsaturated fatty acids (PUFA); in comparison to 78.2%, 14.5% and

7.3%, respectively in *Hermetia illucens* pre-pupae. Lauric acid (LA), oleic acid (OA) and γ-linoleic acid (GLA) were found to be the dominant SFA, MUFA and PUFA in both oil samples.

In contrast, MUFA was the dominant class of FA (48.80 ± 1.19%), followed by PUFA (25.73 ± 4.73%), and SFA (22.22 ± 0.68%) in fish oil. Additionally, C16:0 (palmitic acid) was the main SFA with 13.16 ± 0.20%. Moreover, 42.24 ± 1.73% of C18:1n9(c) (Oleic acid) was found to be predominated in MUFA and 16.33 ± 0.06 % of C18:2n6(c) (linoleic acid) was found to be predominant in PUFA.

Table 4.3: Fatty Acid Composition (Percent Fatty Acid) of *Hermetia illucens* Larvae, Pre-pupae Meal and Fish Oil

Fatty Acid	Larvae meal	Pre-pupae meal	Fish oil	p-value
C10:0	2.29 ± 0.15	1.62 ± 0.14	nd	<0.001
C12:0	57.38 ± 2.45	55.99 ± 1.71	nd	<0.001
C14:0	7.96 ± 0.66	6.68 ± 0.23	2.68 ± 0.13	<0.001
C14:1	0.06 ± 0.02	0.03 ± 0.02	nd	0.006
C15:0	nd	0.01 ± 0.02	0.31 ± 0.06	<0.001
C16:0	11.11 ± 0.27	12.23 ± 0.77	13.16 ± 0.20	0.018
C16:1	3.74 ± 0.10	3.08 ± 0.23	3.48 ± 0.03	0.002
C17:0	0.01 ± 0.01	0.01 ± 0.01	0.50 ± 0.04	<0.001
C17:1	0.16 ± 0.08	0.15 ± 0.10	nd	0.193
C18:0	1.96 ± 0.31	1.60 ± 0.32	4.20 ± 0.29	<0.001
C18:1 n9C	10.07 ± 1.82	11.28 ± 0.93	42.24 ± 1.73	<0.001
C18:2 n6C	0.84 ± 0.12	3.86 ± 0.98	16.33 ± 0.06	0.012
C18:3 n6	nd	nd	0.39 ± 0.11	<0.001
C18:3 n3	4.42 ± 0.63	3.42 ± 1.18	4.57 ± 0.08	0.158
C20:0	nd	nd	0.20 ± 0.02	<0.001
C20:1	nd	nd	2.33 ± 0.04	<0.001
C21:0	nd	0.02 ± 0.03	1.02 ± 0.09	<0.001
C20:3n6	nd	nd	0.89 ± 0.11	<0.001
C20:4n6	nd	nd	0.48 ± 0.14	<0.001
C20:3n3	nd	nd	0.49 ± 0.17	0.002
C22:0	0.01 ± 0.01	0.02 ± 0.01	0.17 ± 0.08	0.020
C22:1n9	nd	nd	0.37 ± 0.17	0.007
C20:5n3	nd	nd	2.53 ± 0.08	<0.001
C22:2	nd	nd	0.22 ± 0.04	<0.001
C24:0	nd	nd	0.06 ± 0.03	0.022
C24:1	nd	nd	0.23 ± 0.06	0.034
C22:6n3	nd	nd	3.16 ± 0.02	<0.001

Table 4.3: (Continued)

Fatty Acid	Larvae meal	Pre-pupae meal	Fish oil	p-value
ΣSFA	80.70 ± 2.67	78.18 ± 1.73	22.22 ± 0.68	<0.001
ΣMUFA	14.03 ± 1.93	14.53 ± 1.21	48.80 ± 1.19	<0.001
ΣPUFA	6.21 ± 0.74	7.28 ± 0.57	25.73 ± 4.73	0.001
Σn3	4.42 ± 0.63	3.42 ± 1.18	10.72 ± 0.13	<0.001
Σn6	0.84 ± 0.12	3.86 ± 0.98	14.80 ± 4.67	0.005

4.2 Formulated Experimental Diets

The experimental diets were formulated to be iso-lipid (approximately 10g/100g crude lipid), iso-protein (approximately 45g/100g crude protein) and iso-energetic (approximately 16 MJ/kg feed). Four experimental diets were designed with increasing inclusion of *Hermetia illucens* pre-pupae meal at 0% (HM0), 4% (HM40), 8% (HM80) and 12% (HM120). The proximate and fatty acid analyses were then carried out.

4.2.1 Proximate Composition of Experimental Diets

The crude lipid, crude protein and gross energy of the diets were comparable, ranging 9.57 – 10.26%, 45.06 – 47.98% and 15.49 – 16.10 MJ/kg, respectively as shown in Table 4.4.

Table 4.4: Proximate Composition of Experimental Diets

Chemical Composition	Experimental Diets				p-value
	HM0	HM40	HM80	HM120	
(% of dry matter)					
Dry matter	83.23 ± 0.21	88.00 ± 0.05	87.21 ± 0.25	92.00 ± 0.04	<0.001
Crude lipid	9.57 ± 0.34	9.88 ± 0.19	10.10 ± 0.22	10.26 ± 0.61	0.360
Chitin	NIL	2.07 ± 0.11	4.35 ± 0.16	7.10 ± 0.08	<0.001
Crude protein*	45.06 ± 0.79	46.94 ± 1.03	47.55 ± 2.98	47.98 ± 1.25	0.058
Gross Energy (MJ kg^{-1})	15.49 ± 0.20	15.63 ± 0.01	16.10 ± 0.02	16.01 ± 0.08	0.001

Note: The data were stated in mean ± standard deviation in triplicate (n=3).
* Crude protein content with chitin correction.

4.2.2 Fatty Acid Profile of Experimental Diets

The FA profile of experimental diets is shown in Table 4.5. In diets containing HI meal, the dominant FAs are SFA (49.66 – 74.88%), followed by MUFA (18.50 – 34.69%) and PUFA (6.12 – 15.66%). Meanwhile, MUFA (41.83%) was the dominant FA class, followed by SFA (38.44%) and PUFA (19.74%) in HM0 without HI meal. It was also noticed that the concentration of total SFA increased at the increase of the HI meal in the diet. Additionally, the concentrations of capric acid (C10:0), lauric acid (C12:0) and myristic acid (C14:0) increased with the increase replacement of fish meal and fish oil. In particular, C12:0 showed a 174-fold higher concentration in HM120 than in HM0. On the other hand, the concentrations of stearic acid (C18:0) decreased noticeably with increased substitution of fish meal by HI meal. Noticeable decreases were observed for total MUFA and PUFA when the portion for insect meal were increased. Omega-3 and omega-6 FAs decreased significantly and reached the lowest concentration in the HM120 diet. Oleic acid (C18:1n9) in

MUFA decreased sharply from 33.59% in HM0 to 11.90% in HM120. Moreover, C20:5n3 (eicosapentaenoic acid, EPA) and C22:6n3 (docosahexaenoic acid, DHA) in PUFA also decreased consistently as more fish oil (EPA and DHA enrich-oil) in the diets were substituted.

Table 4.5: Fatty Acids Composition (Percentage Fatty Acid) of Experimental Diets

Fatty Acids	HM0	HM40	HM80	HM120	p-value
C10:0	nd	0.14 ± 0.10	0.49 ± 0.05	0.60 ± 0.17	0.001
C12:0	0.20 ± 0.10	1.35 ± 1.42	22.49 ± 0.68	34.92 ± 1.23	<0.001
C13:0	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.079
C14:0	3.61 ± .026	5.22 ± 0.23	6.96 ± 0.04	8.87 ± 0.03	<0.001
C14:1	0.02 ± 0.02	0.03 ± 0.01	0.06 ± 0.01	0.11 ± 0.01	<0.001
C15:0	0.59 ± 0.02	0.59 ± 0.02	0.56 ± 0.02	0.57 ± 0.03	0.599
C16:0	22.97 ± 0.69	23.06 ± 0.11	22.32 ± 0.75	22.11 ± 0.40	0.294
C16:1	5.30 ± 0.36	5.37 ± 0.12	5.41 ± 0.07	5.54 ± 0.12	0.700
C17:0	0.94 ± 0.05	0.87 ± 0.02	0.86 ± 0.03	0.77 ± 0.08	0.057
C17:1	0.17 ± 0.03	0.18 ± 0.01	0.14 ± 0.02	0.13 ± 0.02	0.298
C18:0	8.36 ± 0.61	7.92 ± 0.30	7.29 ± 0.09	6.16 ± 0.09	0.001
C18:1 n9T	0.16 ± 0.06	0.16 ± 0.04	0.10 ± 0.01	0.11 ± 0.03	0.234
C18:1 n9C	33.59 ± 0.81	26.95 ± 0.33	19.45 ± 0.50	11.90 ± 0.09	<0.001
C18:2 n6C	10.78 ± 0.38	8.26 ± 0.16	5.58 ± 0.09	2.75 ± 0.03	<0.001
C18:3 n6	0.28 ± 0.05	0.21 ± 0.04	0.12 ± 0.02	0.05 ± 0.02	0.001
C18:3 n3	2.32 ± 0.21	2.09 ± 0.09	1.96 ± 0.06	1.59 ± 0.03	0.002
C20:0	0.32 ± 0.03	0.29 ± 0.01	0.25 ± 0.01	0.23 ± 0.02	0.014
C20:1	1.76 ± 0.09	1.31 ± 0.06	0.80 ± 0.05	0.31 ± 0.02	<0.001
C20:2	0.12 ± 0.01	0.11 ± 0.06	0.14 ± 0.04	0.09 ± 0.02	0.498
C21:0	0.66 ± 0.04	0.50 ± 0.04	0.30 ± 0.01	0.08 ± 0.02	<0.001
C20:3n6	0.57 ± 0.08	0.40 ± 0.03	0.31 ± 0.03	0.20 ± 0.09	0.003
C20:4n6	0.65 ± 0.11	0.60 ± 0.06	0.59 ± 0.06	0.60 ± 0.34	0.990
C20:3n3	0.20 ± 0.02	0.16 ± 0.02	0.11 ± 0.02	0.03 ± 0.01	<0.001
C22:0	0.27 ± 0.04	0.28 ± 0.00	0.28 ± 0.02	0.22 ± 0.00	0.067
C22:1n9	0.23 ± 0.02	0.18 ± 0.02	0.08 ± 0.04	0.07 ± 0.02	0.001
C20:5n3	1.79 ± 0.38	1.42 ± 0.14	1.17 ± 0.12	0.49 ± 0.04	0.002
C23:0	0.16 ± 0.02	0.11 ± 0.05	0.15 ± 0.09	0.11 ± 0.00	0.701
C22:2	0.22 ± 0.10	0.16 ± 0.02	0.05 ± 0.03	0.06 ± 0.02	0.034
C24:0	0.33 ± .02	0.30 ± 0.02	0.26 ± 0.00	0.20 ± 0.01	0.001
C24:1	0.59 ± 0.06	0.51 ± 0.03	0.30 ± 0.15	0.34 ± 0.01	0.022
C22:6n3	2.81 ± 0.68	2.24 ± 0.41	1.37 ± 0.71	0.77 ± 0.06	0.021
ΣSFA	38.44 ± 1.09	49.66 ± 1.29	62.26 ± 0.50	74.88 ± 0.76	<0.001
ΣMUFA	41.83 ± 0.80	34.69 ± 0.32	26.33 ± 0.39	18.50 ± 0.21	<0.001
ΣPUFA	19.74 ± 1.86	15.66 ± 0.98	14.41 ± 0.72	6.12 ± 0.55	<0.001
Σn3	7.11 ± 1.25	5.91 ± 0.65	4.61 ± 0.70	2.88 ± 0.12	0.004
Σn6	12.28 ± 0.55	9.48 ± 0.28	6.61 ± 0.01	3.59 ± 0.44	<0.001

Values are presented in mean ± SD (n=3).

4.3 Outcomes of Feeding Trial

Hermetia illucens pre-pupae meal had higher value of crude protein content and gross energy and slightly lower crude lipid content compared to larvae meal. Moreover, it also contained more MUFA and PUFA. Thus, based on the chemical and fatty acid compositions, pre-pupae meal was chosen as the best alternative insect meal to substitute fish meal and fish oil at different inclusion levels. At the beginning of the feeding trial, the average initial weights of *Oxyeleotris marmorata* juveniles were determined to be 15.61g. The outcomes of experimental diets fed on *Oxyeleotris marmorata* were evaluated based on growth performances, diet utilization efficiency, survival rate, biological indices and biochemical properties of whole body and fillet of *Oxyeleotris marmorata* at the end of the 12 weeks feeding trial.

4.3.1 Growth Performances

Table 4.6, *Oxyeleotris marmorata* fed with HM0 had the lowest weight gain (1.01%), followed by HM120 (1.55%). Meanwhile HM40 and HM80 had significant weight gain of 17.03% and 25.72% respectively. When compared to the feed consumption in grams, the HM0 meal was consumed the least. As the HM content increase, the feed consumption (g) increased as observed in HM40, the fish consumed the most which is 74.29g, for HM80 is 62.6g and 47.13g for HM120 experimental diet. Thus, the specific growth rate (SRG), feed conversion rate (FCR) and also the protein efficiency ratio (PER) exhibited

values corresponding to the feed consumption (g) of the fish. The SGR and PER for HM0, HM40, and HM80 increased and then dropped for the HM120. Meanwhile, for HM0, HM40 and HM80, the PER values declined from 51.21 to 24.12 and then to 15.27, yet higher value of PER was recorded for HM120 dietary meal. The survival rate on the other hand showed fishes fed with HM0 meal was the highest with 80% marble gobies surviving. A downward survival trend is observed with the rate reducing to 60% for HM40, 50% for HM120 and HM80 having the highest mortality with surviving rate of only 40%.

The relatively lower survival rates (40%) amongst the experimental diets could be due to the lower chitobiase level available in the juvenile fish, thereby unable to digest the faecal protein, causing lost in energy and resulting in their death. On the other hand, *Oxyeleotris marmorata* fed with diet HM0 showed the best result in survival rate (80%) but the specific growth rate (0.07 g day^{-1}), feed conversion ratio, 51.21 and protein efficiency (0.042) were the lowest compared to other diets. In the case of HM120 the survival rate is at 50%, which is among the lowest however, still slightly better compared to HM80, the better result may be due to lower feed consumption of fish fed with HM120 feed, which lowers the harmful effects of chitin, explaining the better survival rate, while having an adverse effect on weight gain.

Table 4.6: Growth Performance, Diet Utilization Efficiency and Survival Rate of *Oxyeleotris marmorata* Juveniles Fed with the Experimental Diets

Growth parameter	Experimental diets			
	HM0	HM40	HM80	HM120
Initial weight (g)	15.61	18.09	15.94	16.16
Final weight (g)	16.62	21.17	20.04	16.41
Weight gained (%)	6.47	17.03	25.72	1.55
Feed consumption (g)	51.72	74.29	62.60	47.13
Specific growth rate, SGR	0.07	0.19	0.27	0.02
Feed conversion ratio, FCR	51.21	24.12	15.27	188.52
Protein efficiency ratio, PER	0.042	0.088	0.137	0.011
Survival rate	80	60	40	50

4.3.2 Biological Indices of *Oxyeleotris marmorata*

Table 4.7 shows the biological indices of *Oxyeleotris marmorata* juveniles at the start of feeding trial and after 12 weeks of experimental diets feeding.

The fillet yield, viscerosomatic index and hepatosomatic index of *Oxyeleotris marmorata* juveniles at initial stage was 36.07 ± 1.81%, 10.48 ±1.66% and 3.28 ± 0.51%, respectively. After 12 weeks of experimental diets feeding, fillet yield (36.8 – 37.36%), viscerosomatic index (8.59 – 12.51%) and hepatosomatic (3.07 – 6.85%) were obtained. The largest fillet yield was obtained from the fish fed with HM120 (37.45 ± 2.31) followed by HM40 (37.36 ± 3.65) and HM0 (37.06 ± 3.32) with a slight reduction in fillet yield. Meanwhile, HM80 (36.68 ± 1.11) had the lowerst fillet yield. On the other hand,

VSI and HSI increased from HM 0 (9.42 ± 1.59 and 3.58 ± 0.87) to HM40 (10.74 ± 1.07 and 4.22 ± 0.57) and then increased further to HM80 (12.51 ± 0.65 and 6.85 ± 0.32). However, the VSI and HSI reduced significantly for HM120 (8.59 ± 0.81 and 3.07 ± 0.82, respectively). The fillet yield, VSI and HSI of *Oxyeleotris marmorata* juveniles at initial stage was 36.07 ± 1.81%, 10.48 ±1.66% and 3.28 ± 0.51%, respectively. After 12 weeks of experimental diets feeding, slight increase was observed in the fillet yield of the fish as the *Hermetia illucens* content increased in the feed, for HM40, HM80 and HM120 the fillet yield and rose to 37.06 ± 3.32% , 36.68 ± 1.11% and 37.45 ± 2.31%, respectively. Meanwhile, for the meal with no *Hermetia illucens* content (HM0) had a very similar fillet yield as the initial of 37.06 ± 3.32%. The VI similar to fillet yield recorded an increase from the initial value for HM40 (10.74 ± 1.07%), HM80 (12.51 ± 0.65) and HM120 (8.59 ± 0.81). however, for the fish fed with diet HM0, the VI reduced from the initial 10.48 ± 1.66% to 9.42 ± 1.59%. on the hand, *Hermetia illucens* for HM0 (3.58 ± 0.87%), HM40 (4.22 ± 0.57%) and HM80 (6.85 ± 0.32%) diets all had a positive increment except for HM120 which declined to 3.07 ± 0.8%.

Table 4.7: Biological Indices of *Oxyeleotris marmorata* Fed with Experimental Diets

Parameter	Initial	Experimental diet				p-value
		HM0	HM40	HM80	HM120	
Fillet yield	36.07 ± 1.81	37.06 ± 3.32	37.36 ± 3.65	36.68 ± 1.11	37.45 ± 2.31	0.032
Viscerosomatic index	10.48 ± 1.66	9.42 ± 1.59	10.74 ± 1.07	12.51 ± 0.65	8.59 ± 0.81	0.002
Hepatosomatic index	3.28 ± 0.51	3.58 ± 0.87	4.22 ± 0.57	6.85 ± 0.32	3.07 ± 0.8	0.992

* Parameters in percentage, % of total body weight.

4.3.3 Proximate Composition of Whole Body and Fillet of *Oxyeleotris marmorata*

Table 4.8 shows the biochemical properties of *Oxyeleotris marmorata* juveniles whole body and fillet before the feeding and after 12 weeks experimental diets feeding.

In the beginning, the moisture content, crude lipid, crude protein and ash content of whole body of *Oxyeleotris marmorata* juveniles was 75.83 ± 0.63%, 3.39 ± 0.39%, 79.33 ± 2.06% and 17.30 ± 2.05%, respectively. Moreover, the fillet with moisture (77.43 ± 0.57%), crude lipid (1.92 ± 0.17%), crude protein (18.89 ± 0.26) and ash (1.84 ± 0.23%). After 12 weeks of experimental diets feeding, the whole body of *Oxyeleotris marmorata* juveniles with moisture content, crude lipid, crude protein and ash content were ranging from 74.85 – 76.19%, 3.46 – 6.38%, 76.13 – 79.92% and 15.82 – 18.48%, respectively. However, the fillet dissected from *Oxyeleotris marmorata* juveniles fed with 4 different formulated diets consisted of moisture (76.59 – 77.61%), crude lipid (2.11 – 2.70%), crude protein (16.31 – 18.91%) and Ash (1.69 – 2.34%) were obtained.

Table 4.8: Proximate Composition of Whole Body and Fillet of *Oxyeleotris marmorata* Fed Experimental Diets

Parameter	Initial	Experimental diet				p-value
		HM0	HM40	HM80	HM120	
Whole body*						
Moisture	75.83 ± 0.63	74.99 ± 0.52	74.85 ± 0.43	75.15 ± 0.68	76.19 ± 0.43	0.116
Crude Lipid	3.39 ± 0.39	5.62 ± 1.11	6.38 ± 1.51	4.62 ± 0.70	3.46 ± 1.06	0.134

Table 4.8: (Continued)

Parameter	Initial	Experimental diet				p-value
		HM0	HM40	HM80	HM120	
Crude Protein	79.33 ± 2.06	76.13 ± 0.71	76.71 ± 1.65	79.92 ± 0.41	77.41 ± 1.30	0.042
Ash	17.30 ± 2.05	18.14 ± 1.92	16.94 ± 2.40	15.82 ± 0.86	18.48 ± 2.08	0.522
Fillet[#]						
Moisture	77.43 ± 0.57	77.15 ± 0.75	76.59 ± 0.32	76.65 ± 0.63	77.61 ± 0.82	0.418
Crude Lipid	1.92 ± 0.17	2.11 ± 0.02	2.38 ± 0.11	2.70 ± 0.24	2.19 ± 0.17	0.029
Crude Protein	18.89 ± 0.26	18.91 ± 0.29	18.78 ± 0.32	18.58 ± 0.21	18.31 ± 0.23	0.001
Ash	1.84 ± 0.23	1.69 ± 0.27	2.16 ± 0.19	2.16 ±0.19	2.34 ± 0.33	0.169

***** Parameters for whole body, moisture in wet weight basis; crude lipid, crude protein and ash in dry weight basis.
# All parameters for fillet are in wet weight basis.

4.4 Fatty Acid Composition of *Oxyeleotris marmorata*

The fatty acid profiling was done on whole body, fillet and liver of *Oxyeleotris marmorata* as shown in Table 4.9, Table 4.10 and Table 4.11. In the FA profiling of whole body and liver, SFA was the predominated FA, followed by MUFA and PUFA. On the other hand, the dominant FA in fillet was MUFA followed by SFA, and PUFA.

The predominant FA in whole body of *Oxyeleotris marmorata* was SFA (38.94 – 53.65%), palmitic acid (21.72 – 23.56%) and stearic acid (9.33 – 14.98%) were two most abundant SFA while palmitoleic acid (4.26 – 7.49%) and oleic acid (17.40 – 26.07%) were two major MUFA (24.08 – 35.52%).

Meanwhile, the total PUFA was ranged from 22.26% to 25.54%, 4 of its major FA consisted of linoleic acid (4.47 – 8.52%), arachidonic acid (2.98 – 5.30%), eicosapentaenoic acid (1.22 – 2.16%) and docosahexaenoic acid (7.79 – 9.19%).

Based on *Oxyeleotris marmorata* juvenile's fillet, palmitic acid (23.23 – 25.42%) and stearic acid (12.94 – 14.90%) were dominated in SFA (44.88 – 52.94%), while palmitic acid (3.47 – 5.12%) and stearic acid (14.94 – 19.45%) were dominated in MUFA (21.16 – 26.35%). As PUFA was predominated compared to MUFA in fillet, plenty of PUFAs were detected such as linoleic acid (5.28 – 7.53%), α-linoleic acid (1.09 – 1.50%), arachidonic acid (4.57 – 5.76%), eicosapentaenoic acid (1.68 – 2.29%) and docosahexaenoic acid (9.31 – 11.69%).

Similar to those observed in whole body, the main type of FA in the liver of *Oxyeleotris marmorata* juveniles was exactly the same (ΣSFA > ΣMUFA > ΣPUFA). Hence, the major FAs such as myristic acid (5.59 – 10.42%), palmitic acid (27.68 – 32.72%) and stearic acid (6.23 – 6.92%) were detected in SFA (43.42- 58.84%). Furthermore, second class of FA was MUFA ranged from 30.59% to 43.45%, majority formed by palmitoleic acid (8.92 – 13.85%) and oleic acid (19.67 – 27.56%). At the lowest content was PUFA (10.57 – 13.13%) which consisted of linoleic acid (6.47 – 8.14%), α-linoleic acid (1.28 – 1.58%) and docosahexaenoic acid (1.16 – 1.94%).

Besides that, when comparing the fatty acid composition of the experimental diet to the whole body, the SFA for HM0 (38.44 ± 1.09%) and 38.94 ± 0.03% for the whole body had almost identical values. However, the values of SFA build-up in whole body of the fish fed with diet HM40, HM80 and HM120 having 49.66 ± 1.29%, 62.26 ± 0.50% and 74.88 ± 0.76% SFA content, respectively, are higher than that of the respective experimental diet HM40 (41.42 ± 0.09%), HM80 (47.02 ± 0.19%) and HM120 (53.65 ± 0.61%). Meanwhile for MUFA, a similar pattern is seen where lesser MUFA is accumulated in the whole body of *Oxyeleotris marmorata* with significant difference in all diets, 35.52 ± 0.07% , 33.32 ± 0.08%, 29.78 ± 0.08% and 24.08 ± 0.86% compared to the original 41.83 ± 0.80%, 34.69 ± 0.32%, 26.33 ± 0.39% and 18.50 ± 0.21% MUFA content of the experimental diets itself. On the other hand, substantially saw an increase in whole body PUFA in comparison to the PUFA values of HM diets itself, where for HM0 the value increased from 19.74 ± 1.86% to as high as 25.54 ± 0.04%, for HM40 the value of PUFA rose from 15.66 ± 0.98% to 25.26 ± 0.16%, HM80 value saw increment of 14.41 ± 0.72% to 23.20 ± 0.27% and the PUFA values of HM120 also improved from 6.12 ± 0.55% to 22.26 ± 0.26%.

The SFA value for fillet of fish fed HM0 was higher compared to the SFA content of diet HM0 itself rising from 38.44 ± 1.09% to 44.88 ± 0.35%. HM40 diet and the fillet of fish fed HM40 had similar PUFA values of around 49.06 ± 0.07% and 49.06 ± 0.07%. Meanwhile, the values of SFA for the HM diets for, HM80 (47.02 ± 0.19%) had lower levels compared to the SFA build-up in whole body of the fish with diet HM80 having 47.94 ± 0.11%. However,

HM120 (53.65 ± 0.61%) had higher levels compared to the SFA build-up in whole body of the fish with diet HM120 having only 52.94 ± 0.02% SFA content.

Besides that, HM0 (41.83 ± 0.80%), HM40 (34.69 ± 0.32%) and HM80 (26.33 ± 0.39%) feeds produced fillets with lower MUFA content, where-by, HM0 fed fillet accumulated MUFA value of 26.35 ± 0.26%, 23.59 ± 0.09% MUFA accumulated by HM40 fillet and 25.30 ± 0.05% accumulated in fillet fed by HM80. Only HM120 fed fillet had MUFA content of 21.16 ± 0.07 % which is higher compared to the HM120 feed MUFA content itself (18.50 ± 0.21%). On the other hand, substantially saw an increase in fillet PUFA in comparison to the PUFA values of HM diet itself, where for HM0 the value increased from 19.74 ± 1.86% to as high as 28.77 ± 0.59, for HM40 the value of PUFA rose from 15.66 ± 0.98% to 27.35 ± 0.12%, HM80 value saw increment of 14.41 ± 0.72% to 26.76 ± 0.09% and the PUFA values of HM120 also improved from 6.12 ± 0.55% to 25.90 ± 0.05%.

SFA content of the HM0 (38.44 ± 1.09%) and HM80 (49.66 ± 1.29%) feed produced fish livers with higher SFA values of 43.42 ± 2.59% and 50.69 ± 3.47% respectively, while HM80 (62.26 ± 0.50%) and HM120 (74.88 ± 0.76%) feed produced livers with lower SFA content (49.90 ± 2.4% and 58.84 ± 2.36%, respectively). Contrastingly to SFA, MUFA content of the HM0 (38.44 ± 1.09%) and HM80 (49.66 ± 1.29%) feed produced fish livers with lower MUFA values of 43.45 ± 1.41% and 38.38 ± 2.57%, correspondingly, while, HM80 (62.26 ± 0.50%) and HM120 (74.88 ± 0.76%) feed produced livers with higher MUFA content (37.75 ± 3.19% and 30.59 ± 3.11%, correspondingly). Last but

least, reduction was observed in PUFA content accumulation in livers for fish fed with HM0 (from 19.74 ± 1.86% to 13.13 ± 2.01%), HM40 (from 15.66 ± 0.98% to 10.93 ± 2.25%) and HM80 (from 14.41 ± 0.72% to 12.35 ± 0.87%). However, for liver of fish fed on HM120 diet accumulated smaller PUFA content (10.57 ± 1.82 %) matched to the PUFA content of 6.12 ± 0.55% of the HM120 diet itself.

Table 4.9: Whole Body Fatty Acid Composition (Percentage of Total Fatty Acid) of *Oxyeleotris marmorata* Fed Experimental Diets

Fatty Acid	Initial	HM0	HM40	HM80	HM120	p-value
C10:0	0.05 ± 0.00	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.14 ± 0.00	<0.001
C12:0	0.61 ± 0.02	0.32 ± 0.00	0.97 ± 0.01	6.02 ± 0.07	5.07 ± 0.05	<0.001
C14:0	3.86 ± 0.03	3.31 ± 0.04	3.86 ± 0.02	6.07 ± 0.04	5.12 ± 0.05	<0.001
C15:0	0.66 ± 0.02	0.61 ± 0.00	0.63 ± 0.00	0.57 ± 0.00	0.46 ± 0.01	<0.001
C16:0	19.91± 0.05	21.72 ± 0.02	23.28 ± 0.07	22.33 ± 0.03	23.56 ± 0.26	<0.001
C16:1	8.18 ± 0.01	7.11 ± 0.02	7.49 ± 0.02	6.97 ± 0.08	4.26 ± 0.05	<0.001
C17:0	0.84 ± 0.04	0.80 ± 0.00	0.80 ± 0.00	0.77 ± 0.06	0.68 ± 0.01	0.019
C17:1	0.23 ± 0.01	0.21 ± 0.01	0.20 ± 0.00	0.08 ± 0.01	0.07 ± 0.02	<0.001
C18:0	10.69 ± 0.09	9.94 ± 0.03	9.76 ± 0.03	9.33 ± 0.03	14.98 ± 0.22	<0.001
C18:1n9T	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.00	0.14 ± 0.01	0.375
C18:1n9C	20.87± 0.06	26.07 ± 0.04	23.68 ± 0.06	21.04 ± 0.05	17.40 ± 0.93	<0.001
C18:2n6C	7.26 ± 0.01	8.52 ± 0.01	7.91 ± 0.17	7.38 ± 0.20	4.47 ± 0.06	<0.001
C18:3n6	0.49 ± 0.04	0.37 ± 0.01	0.36 ± 0.01	0.30 ± 0.01	0.19 ± 0.01	<0.001
C18:3n3	1.59 ± 0.01	1.57 ± 0.00	1.55 ± 0.00	1.91 ± 0.01	1.34 ± 0.02	<0.001
C20:0	0.27 ± 0.01	0.25 ± 0.00	0.22 ± 0.00	0.22 ± 0.00	0.32 ± 0.01	<0.001
C20:1	0.79 ± 0.02	1.00 ± 0.00	0.91 ± 0.01	0.76 ± 0.00	0.36 ± 0.01	<0.001
C20:2	0.11 ± 0.02	0.09 ± 0.00	0.09 ± 0.00	0.10 ± 0.00	0.12 ± 0.00	<0.001
C21:0	0.43 ± 0.00	0.53 ± 0.0	0.52 ± 0.00	0.41 ± 0.00	0.23 ± 0.01	<0.001
C20:3n6	0.98 ± 0.11	0.75 ± 0.01	0.76 ± 0.02	0.61 ± 0.01	0.87 ± 0.01	<0.001
C20:4n6	4.77 ± 0.07	3.24 ± 0.01	2.98 ± 0.03	3.08 ± 0.01	5.30 ± 0.06	<0.001
C20:3n3	0.15 ± 0.02	0.18 ± 0.00	0.19 ± 0.01	0.15 ± 0.01	0.10 ± 0.01	<0.001
C22:0	0.28 ± 0.01	0.23 ± 0.00	0.22 ± 0.00	0.21 ± 0.00	0.46 ± 0.01	<0.001
C22:1n9	0.35 ± 0.01	0.29 ± 0.00	0.27 ± 0.01	0.25 ± 0.00	0.58 ± 0.01	<0.001
C20:5n3	2.86 ± 0.01	2.16 ± 0.01	2.07 ± 0.01	1.74 ± 0.01	1.22 ± 0.02	<0.001
C23:0	0.17 ± 0.00	0.16 ± 0.00	0.15 ± 0.01	0.13 ± 0.00	0.34 ± 0.00	<0.001
C22:2	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.00	0.13 ± 0.00	0.15 ± 0.00	0.005
C24:0	1.30 ± 0.01	0.96 ± 0.00	0.91 ± 0.01	0.87 ± 0.00	2.21 ± 0.02	<0.001
C24:1	0.74 ± 0.00	0.73 ± 0.01	0.65 ± 0.01	0.55 ± 0.01	1.28 ± 0.04	<0.001
C22:6n3	11.23 ± 0.03	8.50 ± 0.02	9.19 ± 0.04	7.79 ± 0.07	8.51 ± 0.12	<0.001
ΣSFA	39.14 ± 0.04	38.94 ± 0.03	41.42 ± 0.09	47.02 ± 0.19	53.65 ± 0.61	<0.001
ΣMUFA	31.28 ± 0.03	35.52 ± 0.07	33.32 ± 0.08	29.78 ± 0.08	24.08 ± 0.86	<0.001
ΣPUFA	29.58 ± 0.06	25.54 ± 0.04	25.26 ± 0.16	23.20 ± 0.27	22.26 ± 0.26	<0.001
Σn3	15.83 ± 0.05	12.41 ± 0.03	13.00 ± 0.04	11.60 ± 0.06	11.16 ± 0.14	<0.001
Σn6	13.49 ± 0.09	12.87 ± 0.00	12.02 ± 0.14	11.37 ± 0.21	10.82 ± 0.12	<0.001

Values are presented in mean ± SD (n=3).

Table 4.10: Fillet Fatty Acid Composition (Percentage of Total Fatty Acid) of Initial and Final *Oxyeleotris marmorata* Juveniles Fed Experimental Diets

Fatty Acid	Initial	HM0	HM40	HM80	HM120	p-value
C10:0	0.15 ± 0.01	0.21 ± 0.02	0.20 ± 0.01	0.10 ± 0.02	0.28 ± 0.02	<0.001
C12:0	1.79 ± 0.04	2.46 ± 0.08	4.61 ± 0.01	3.13 ± 0.03	4.60 ± 0.01	<0.001
C14:0	2.94 ± 0.08	2.48 ± 0.10	2.75 ± 0.05	3.93 ± 0.02	3.70 ± 0.05	<0.001
C15:0	0.70 ± 0.10	0.57 ± 0.05	0.54 ± 0.03	0.55 ± 0.01	0.54 ± 0.01	0.758
C16:0	23.21 ± 0.13	23.23 ± 0.17	24.44 ± 0.05	24.75 ± 0.08	25.42 ± 0.06	<0.001
C16:1	5.22 ± 0.03	4.33 ± 0.05	3.47 ± 0.01	5.12 ± 0.01	3.66 ± 0.01	<0.001
C17:0	0.83 ± 0.02	0.67 ± 0.03	0.79 ± 0.01	0.80 ± 0.01	0.81 ± 0.02	<0.001
C17:1	0.26 ± 0.02	0.24 ± 0.07	0.26 ± 0.02	0.20 ± 0.00	0.29 ± 0.05	0.360
C18:0	13.26 ± 0.02	12.94 ± 0.09	13.40 ± 0.05	12.56 ± 0.02	14.90 ± 0.03	<0.001
C18:1n9T	0.16 ± 0.03	0.15 ± 0.03	0.15 ± 0.03	0.13 ± 0.01	0.13 ± 0.01	0.551
C18:1n9C	17.85± 0.10	19.45 ± 0.19	17.54± 0.04	18.02 ± 0.01	14.94 ± 0.05	<0.001
C18:2n6C	6.07 ± 0.05	7.01 ± 0.05	7.53 ± 0.01	6.97 ± 0.03	5.28 ± 0.01	<0.001
C18:3n6	0.36 ± 0.03	0.36 ± 0.02	0.26 ± 0.02	0.29 ± 0.01	0.21 ± 0.03	0.001
C18:3n3	1.12 ± 0.03	1.09 ± 0.02	1.21 ± 0.00	1.50 ± 0.01	1.21 ± 0.01	<0.001
C20:0	0.24 ± 0.00	0.23 ± 0.00	0.21 ± 0.00	0.21 ± 0.00	0.27 ± 0.01	<0.001
C20:1	0.76 ± 0.04	0.81 ± 0.06	0.69 ± 0.03	0.65 ± 0.02	0.43 ± 0.02	<0.001
C20:2	0.09 ± 0.02	0.11 ± 0.03	0.09 ± 0.00	0.09 ± 0.00	0.10 ± 0.01	0.600
C21:0	0.53 ± 0.01	0.59 ± 0.02	0.62 ± 0.01	0.51 ± 0.01	0.34 ± 0.02	<0.001
C20:3n6	0.81 ± 0.08	0.91 ± 0.02	1.24 ± 0.05	0.81 ± 0.02	1.00 ± 0.01	<0.001
C20:4n6	5.45 ± 0.05	5.38 ± 0.04	5.26 ± 0.06	4.57 ± 0.04	5.76 ± 0.04	<0.001
C20:3n3	0.21 ± 0.02	0.21 ± 0.07	0.14 ± 0.03	0.16 ± 0.03	0.08 ± 0.01	0.070
C22:0	0.23 ± 0.00	0.26 ± 0.02	0.22 ± 0.01	0.21 ± 0.00	0.33 ± 0.01	<0.001
C22:1n9	0.51 ± 0.03	0.52 ± 0.03	0.53 ± 0.02	0.47 ± 0.02	0.68 ± 0.01	<0.001
C20:5n3	2.44 ± 0.05	1.86 ± 0.87	2.20 ± 0.01	2.29 ± 0.02	1.68 ± 0.02	0.497
C23:0	0.15 ± 0.02	0.16 ± 0.02	0.18 ± 0.01	0.15 ± 0.01	0.24 ± 0.01	0.002
C22:2	0.14 ± 0.01	0.14 ± 0.04	0.11 ± 0.02	0.12 ± 0.00	0.13 ± 0.05	0.748
C24:0	1.04 ± 0.02	1.08 ± 0.02	1.10 ± 0.00	1.02 ± 0.00	1.51 ± 0.01	<0.001
C24:1	0.65 ± 0.01	0.86 ± 0.01	0.94 ± 0.02	0.71 ± 0.01	1.02 ± 0.04	<0.001
C22:6n3	12.83 ± 0.12	11.69 ± 0.19	9.31 ± 0.10	9.94 ± 0.06	10.45 ± 0.11	<0.001
ΣSFA	45.07 ± 0.08	44.88 ± 0.35	49.06 ± 0.07	47.94 ± 0.11	52.94 ± 0.02	<0.001
ΣMUFA	25.42 ± 0.09	26.35 ± 0.26	23.59 ± 0.09	25.30 ± 0.05	21.16 ± 0.07	<0.001
ΣPUFA	29.51 ± 0.17	28.77 ± 0.59	27.35 ± 0.12	26.76 ± 0.09	25.90 ± 0.05	<0.001
Σn3	16.59 ± 0.09	14.85 ± 0.66	12.87 ± 0.13	13.89 ± 0.06	13.43 ± 0.12	0.112
Σn6	12.68 ± 0.09	13.06 ± 0.08	14.29 ± 0.02	12.65 ± 0.03	12.25 ± 0.06	<0.001

Values are presented in mean ± SD (n=3).

Table 4.11: Liver Fatty Acid Composition (Percentage of Total Fatty Acid) of Initial and Final *Oxyeleotris marmorata* Juveniles Fed Experimental Diets

Fatty Acids	Initial	HM0	HM40	HM80	HM120	p-value
C10:0	nd	nd	0.04 ± 0.01	0.02 ± 0.02	0.05 ± 0.01	0.006
C12:0	0.10 ± 0.02	0.17 ± 0.05	3.57 ± 2.07	4.17 ± 1.31	5.93 ± 1.84	0.031
C14:0	4.29 ± 0.95	5.59 ± 0.50	6.88 ± 1.71	6.87 ± 1.21	10.42 ± 1.11	0.020
C14:1	0.22 ± 0.04	0.24 ± 0.02	0.23 ± 0.03	0.20 ± 0.03	0.27 ± 0.03	0.160
C15:0	0.85 ± 0.10	0.91 ± 0.08	0.80 ± 0.10	0.76 ± 0.04	0.75 ± 0.09	0.255
C16:0	30.53 ± 1.54	27.68 ± 2.13	31.22 ± 1.56	29.22 ± 0.06	32.72 ± 1.37	0.041
C16:1	9.75 ± 1.22	13.85 ± 0.78	9.94 ± 0.66	9.37 ± 0.73	8.92 ± 2.10	0.014
C17:0	1.01 ± 0.02	0.96 ± 0.06	0.87 ± 0.06	0.91 ± 0.07	0.86 ± 0.06	0.429
C17:1	0.17 ± 0.05	0.15 ± 0.02	0.12 ± 0.01	0.13 ± 0.04	0.18 ± 0.07	0.593
C18:0	6.83 ± 0.53	6.92 ± 0.17	6.23 ± 0.31	6.87 ± 0.66	6.79 ± 1.64	0.860
C18:1n9T	0.15 ± 0.00	0.11 ± 0.03	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.07	0.728
C18:1n9C	32.24 ± 0.53	27.56 ± 0.69	26.56 ± 1.81	26.61 ± 3.50	19.68 ± 1.42	0.020
C18:2n6C	7.83 ± 0.93	8.14 ± 0.75	7.18 ± 1.15	7.30 ± 0.36	6.47 ± 1.35	0.448
C18:3n6	0.18 ± 0.04	0.29 ± 0.04	0.18 ± 0.07	0.23 ± 0.05	0.14 ± 0.03	0.116
C18:3n3	1.07 ± 0.22	1.28 ± 0.23	1.31 ± 0.37	1.58 ± 0.25	1.29 ± 0.11	0.623
C20:0	0.24 ± 0.02	0.24 ± 0.01	0.23 ± 0.03	0.22 ± 0.03	0.33 ± 0.13	0.394
C20:1	1.28 ± 0.10	1.13 ± 0.04	1.02 ± 0.13	0.95 ± 0.13	0.70 ± 0.09	0.017
C20:2	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.15 ± 0.05	0.055
C21:0	0.38 ± 0.02	0.49 ± 0.09	0.38 ± 0.06	0.41 ± 0.04	0.29 ± 0.05	0.090
C20:3n6	0.22 ± 0.04	0.30 ± 0.10	0.28 ± 0.14	0.28 ± 0.01	0.26 ± 0.05	0.984
C20:4n6	0.23 ± 0.08	0.38 ± 0.11	0.27 ± 0.09	0.39 ± 0.08	0.27 ± 0.05	0.333
C20:3n3	0.10 ± 0.01	0.12 ± 0.02	0.12 ± 0.03	0.15 ± 0.02	0.14 ± 0.02	0.562
C22:0	0.13 ± 0.03	0.13 ± 0.00	0.13 ± 0.02	0.13 ± 0.04	0.23 ± 0.07	0.088
C22:1n9	0.19 ± 0.06	0.15 ± 0.01	0.15 ± 0.02	0.12 ± 0.04	0.21 ± 0.10	0.463
C20:5n3	0.24 ± 0.15	0.46 ± 0.16	0.23 ± 0.11	0.44 ± 0.13	0.24 ± 0.05	0.169
C23:0	0.09 ± 0.05	0.11 ± 0.03	0.13 ± 0.03	0.11 ± 0.04	0.12 ± 0.02	0.910
C22:2	0.14 ± 0.04	0.14 ± 0.11	0.13 ± 0.02	0.12 ± 0.03	0.18 ± 0.10	0.745
C24:0	0.16 ± 0.03	0.21 ± 0.02	0.19 ± 0.03	0.19 ± 0.01	0.31 ± 0.14	0.330
C24:1	0.29 ± 0.05	0.26 ± 0.01	0.25 ± 0.01	0.23 ± 0.05	0.47 ± 0.20	0.161
C22:6n3	0.98 ± 0.33	1.94 ± 0.70	1.16 ± 0.57	1.79 ± 0.51	1.44 ± 0.41	0.535
ΣSFA	44.66 ± 1.18	43.42 ± 2.59	50.69 ± 3.47	49.90 ± 2.41	58.84 ± 2.36	0.004
ΣMUFA	44.28 ± 0.66	43.45 ± 1.41	38.38 ± 2.57	37.75 ± 3.19	30.59 ± 3.11	0.009
ΣPUFA	11.06 ± 1.45	13.13 ± 2.01	10.93 ± 2.25	12.35 ± 0.87	10.57 ± 1.82	0.493
Σn3	2.38 ± 0.68	3.80 ± 1.11	2.82 ± 1.07	3.96 ± 0.90	3.11 ± 0.35	0.569
Σn6	8.46 ± 0.96	9.11 ± 0.93	7.90 ± 1.32	8.20 ± 0.29	7.14 ± 1.37	0.383

Values are presented in mean ± SD (n=3).

CHAPTER 5

DISCUSSIONS

5.1 Biochemical Composition of *Hermetia illucens* Larvae and Pre-pupae

In general, the biochemical composition of *Hermetia illucens* larvae farmed in this study was similar to reported findings. The moisture content of *Hermetia illucens* larvae was slightly higher than pre-pupae in this study. Similar observation was reported by Liu et al. (2016) whereby *Hermetia illucens* larvae contained lower dry matter (higher moisture content) compared to pre-pupae. The insect fluid also known as moisture content of insect maintains body condition and metabolism of the insect activities from ambient factors (Mellanby, 1958). The moisture content of an insect can be affected by the moisture of substrate intake. However, the substrate intake and rearing conditions are the same throughout the *Hermetia illucens* farming process. The different of moisture content can be explained by the morphological changes of larval transformation. Additionally, the presence of high level of other biochemical compositions such as crude protein and crude lipid in the insect biomass resulted in low moisture content (Studier and Sevick, 1992).

The crude lipid of *Hermetia illucens* larvae was slightly higher than that

in pre-pupae similar to those reported by Liu et al (2016). The high lipid content proved that the *Hermetia illucens* larvae was able to assimilate the nutrients uptake from fruit waste while growing and store the energy as lipid form in its biomass (Leong, et al., 2016). However, a slight decrease in lipid content was observed from larval stage to pre-pupal stage; which happened to other insects as well (Wimer and Lumb, 1967; Arrese and Soulages, 2010; Ghosh, Jung and Meyer-Rochow, 2016). This can be explained by the hormonal activity which change the excretion of different hormones at different larval stages (Mirth, et al., 2014). Before the pupation process happens, the juvenile hormone (JH) which excreted by corpus allatum in larvae's brain will be extremely low which inhibit the ingestion of larvae (Kang, et al., 2019; Kaleka, Kaur and Bali, 2019). Afterwards, the process of pupation take place and the prothoracic glands release 20-hydroxyecdysone (20E) which trigger by prothoracicotropic hormone (PTTH) for metamorphosis (Kang, et al., 2019; Kaleka, Kaur and Bali, 2019). However, crude lipid content could be increased throughout the larval development as well. Rachmawati, et al. (2010) and Bosch et al. (2014) reported the crude lipid content of *Hermetia illucens* larvae increased from 12.8% and 23.94% to 19.7% and 27.5%, respectively at pre-pupae state.

Several studies reported the crude lipid accumulation of *Hermetia illucens* larvae (12.8 – 39.2%) and pre-pupae (12.00 – 44.46%) was easily affected by the organic waste ingested (Rachmawati, et al. 2010; Nyugen, Tomberlin and Vanlaerhoven, 2015; Shumo, et al., 2019) such as cattle feces and urine slurry (Newton, et al., 1997), pig manure (Newton, et al., 2005), palm

kernel meal (Rachmawati, et al., 2010), restaurant solid waste with rice straw (Zheng, et al., 2012), poultry feed, pig liver, fish rendering (Nyugen, Tomberlin and Vanlaerhoven, 2015), sewage sludge, palm decanter, fruit waste (Leong, et al., 2015), Trisperma seeds (Abduh, et al., 2018), chicken feed (Spranghers, et al., 2018), chicken manure, kitchen waste, spent grain (Shumo, et al. 2019), wheat bran, fermented maize straw (Gao, et al., 2019). Interestingly, the crude lipid contents of both larvae and pre-pupae farmed in this project were found to be higher than other reported values. The *Hermetia illucens* larvae in this study was reared on fruit waste mainly contained jackfruits, papayas, bananas, mangoes and guavas which were high sugar (carbohydrate) and low in protein content. Handke, et al. (2013) and Pimentel, et al. (2017) explained the larvae converted carbohydrate into body fat (lipids) from sugary (high carbohydrate) major diet. Barragan-Fonseca (2018) further explained *Hermetia illucens* larvae produced more lipids from high-carbohydrate/ low-protein diet compared to well-balanced carbohydrate-protein diet. Same results were reported by Spranghers et al. (2017) i.e. *Hermetia illucens* pre-pupae reared on vegetable waste (45% of carbohydrate) and biogas digestate (7% of carbohydrate) contained 37% and 21% of lipids, respectively.

Even though pre-pupae contained higher degree of chitin which contributed to non-protein nitrogen (N) content as compared to larvae, but the chitin corrected protein content was still higher than that of larvae. The changes of crude protein from larvae to pre-pupae stage can be explained by the physiological changes and hormonal activities happened in larvae body

(Truman and Riddiford, 2002). During the larval – pupal phase, insect larvae generate protein enzymes such as catalase and superoxide dismutase (SOD) which largely found in the molting fluid for the preparation of metamorphosis. Besides this, protease and catalase (both molting enzymes) are enriched between newly grow cuticles and old cuticles (Zhang, et al., 2014). As mentioned in crude lipid content, the crude protein in *Hermetia illucens* larvae varied and affected by the rearing substrates as well. Larvae reared on protein-rich diet assimilated higher protein content but low in lipid content (Oonincx, van Huis and van Loon, 2015; Tinder, et al., 2017). However, it was also affected by the reflux of lipid and protein metabolism which was noticed and mentioned by Liu, et al. (2017). The crude protein of *Hermetia illucens* reported ranged from 39.1% to 56.1% in larvae (Bosch, et al., 2014; Shumo, et al., 2019; Lalander, et al., 2019; Goa, et al., 2019); and 40.88% to 52.1% in pre-pupae (Newton, et al., 2005; Bosch, et al., 2014; Abduh, et al., 2018; Spranghers, et al., 2018). The results of crude protein obtained in this study were within the range of reported findings.

Overall, the properties of *Hermetia illucens* larvae and pre-pupae oil were within the FAO quality standards for fish oil. Accordingly to FAO standard, the AV for fish oil must equal or lesser than 3.0 mgKOH/kg; PV standard of fish oil must equal and lesser 5 mEq/kg (FAO/WHO, 2017).

The class of FA was dominated by SFA (larvae, 80.70 ± 2.67%; pre-pupae, 78.18 ± 1.73%), followed by MUFA (larvae, 14.03 ± 1.93%; pre-pupae,

14.53 ± .21%) and the least was PUFA (larvae, 6.21 ± 0.74%; pre-pupae, 7.28 ± 0.57%). Similar results of FA classes were reported by Makkar, et al. (2014), Liu, et al. (2016), Jucker, et al. (2017), Moula, et al. (2018), Shumo, et al. (2019) and Ewald, et al. (2020), whereby the SFA, MUFA and PUFA were ranging from 26.0 – 75.6%, 11.4 – 32.1% and 3.5 – 35.0% in larvae; 56.5 – 86.0%, 8.8 – 27.2% and 2.8 – 24.1% in pre-pupae, respectively. Additionally, the trend of the FA class mostly predominated by SFA, followed by MUFA, and the least was PUFA were observed. SFA in *Hermetia illucens* meals were approximately 4-fold higher than fish oil. The SFA of the *Hermetia illucens* larvae and pre-pupae mainly composed of C12:0 (lauric acid), followed by C16:0 (palmitic acid) and C14:0 (myristic acid) in the result. Presence of exceptionally high SFA was mainly due to *Hermetia illucens* larvae was able to biosynthesise these SFA *de novo* by utilizing the carbohydrate source from its rearing substrates with the help of *fatty acid synthase* (FAS) and *Acetyl-CoA carboxylase* (ACC) in its body (Stanley-Samuelson, et al., 1985; Beenakkers, van Der Horst and van Marrewijk, 1985; Giron and Casas, 2003; Giannetto, et al., 2020). In accordance to Fast (1970) SFA of dipteran insect should be predominated by C16:0. However, SFA profiling of *Hermetia illucens* larvae and pre-pupae showed highest distribution of C12:0. This trend was observed and mentioned by Oonincx, et al. (2015b). This phenomena was explained as *Hermetia illucens* larvae highly biosynthesised *de novo* SFA (mainly C12:0) which served as energy storage are less subjected to oxidation compared other C16:0 (Marusich, et al., 2020). Furthermore, formation of C10:0, C12:0 and C14:0 can be justified and explained by the action of an additional *thioesterase II* (cytosolic thioesterase) and/ or partial peroxisomal β-oxidation of retroconversion (Knudsen, Clark and

Dils, 1976; Libertini and Smith, 1978, Rioux and Legrand, 2001). Additionally, C16:0 can be elongated to C18:0 (long–chained SFA) through fatty acid elongase 6 (ELOVL 6) (Piccinin, et al., 2019). Moreover, C16:0 and C18:0 undergo further elongation reaction to C16:1 and C18:1n9(c) with the presence of Δ-9-desaturase (Aljohani, et al., 2019).

Other than the mechanism of fatty acids synthesis, dietary substrate of *Hermetia illucens* larvae also plays an important role (Oonincx, et al. 2015b). Different distribution of SFA was observed in larvae reared on different dietary substrate such as food waste (Ewald, et al., 2020), kitchen waste (Shumo, et al., 2019), cow dung (Makkar, et al., 2014), horse manure (Moula, et al., 2018), swine manure (St-Hilaire, et al., 2007), commercial broiler chicken feed (Liu, et al., 2016) and wheat bran (Goa, et al., 2019). The range of C12:0 (7.1 – 53.9% in larvae, 25.0 – 62.5% in pre-pupae), C14:0 (2.3 – 9.5% in larvae, 5.4 – 9.9% in pre-pupae) and C16:0 (8.8 – 22.0% in larvae, 7.2 – 15.4% in pre-pupae) were observed as mentioned in literature review (St-Hilaire, et al., 2007; Spranghers, et al., 2016; Jucker, et al., 2017; Liland, et al., 2017; Moula, et al., 2018).

In the present study, the abundance of palmitoleic and oleic acids in *Hermetia illucens* larvae oil was either synthesised *de novo* or bio-accumulated from dietary substrates (Hoc, et al., 2020). In the synthesis *de novo* mechanism, C16:0 and C18:0 in SFA are desaturated with the presence of Δ-9-desaturase into C16:1 and C18:1n9(c) (Keith, 1967; Wang, et al., 1982; Zeng, et al., 2019).

This was proven by (Hoc, et al., 2020) as the dietary substrate used was extremely low in C16:1 (<0.02%) but the amount of C16:1 found was significantly in pre-pupae oil harvested.

The inclusion of *Hermetia illucens* pre-pupae meal in the experimental diets has an overall positive impact on quality of the feed when considering the increase in the nutritional content especially for crude lipid and protein which increases as *Hermetia illucens* content increase in the feed. Protein and lipid is a source of energy for the fish. However, at a certain level of *Hermetia illucens* pre-pupae meal in feed causes the faecal protein levels to increase as dietary chitin increases redusing the digestibility of the feed thus reducing energy per feed conversion (protein to energy). Similar results with similar trends were reported by Hu (2017), Belghit (2018), Dumas (2018) and Fisher (2020). In simple, the *Hermetia illucens* pre-pupae meal inclusion in the feed is beneficial in a dose-dependent manner. Within a specific range, chitin has a constructive effect on the growth as perceived with HM40 and HM80 showing a significant yet positive growth compared to HM0 which had no dietary chitin. This may be due to gastrointestinal microbiota that increases gut health beneficial in maintaining the good condition of the fish. According to Dumas (2018) chitin at a certain level tend to disrupt the weight gain, feed conversion and lipid digestibility reducing the apparent digestibility (ADC) of protein (Karlsen, et al., 2015) as witnessed by low protein efficiency ratio (PER) for HM120 diet. Chitin in general known to be an immune modulator in fish – ensuring normal function while actively boosting the immune system, besides that, chitin also

helps in absorption of proline and hydroxyproline by promoting prolidase activity a key hydrolysis enzyme found in the small intestine of fish (Wu et al., 2011). Crude protein is one of the facts responsible for providing the fish with the energy for survival. The fruitful high weight gain and high Specific Growth Rate (SGR) for HM40 and HM80 formulated diets.

High amounts of dietary chitin signifies greater faecal protein, which makes it difficult to be hydrolysed and thus lowering the energy per pellet rate conversion causing the fish to ingest more pellet in-order to gain required energy for daily metabolism, sinking the growth rate (Karlsen, et al., 2015). In addition to that, fact that chitin being difficult to digest also explains lower survival rate for fish fed with experimental diets.

Crude protein is perceived as the main growth inhibitor which in the case insect protein has high amounts of lauric acid and also dietary chitin as discussed by Lee, et al., (2020). The abundant weight gain and the high SGR for HM40 and HM80 formulated diet might perhaps be a result of dietary chitin. Chitin in general is known to be an immune modulator in fish which ensures a normal immune function while also actively boosting the immune system of a fish. Dietary chitin also to an extent helps in absorption of proline and hydroxyproline in the small intestine (Tippayadara, et al., 2021), which is done by promoting prolidase activity a key hydrolysis enzyme promoting gut health. Thus, increasing the digestibility and absorption of the feed and its nutrients.

In contrast to that, HM120 on the other hand had apparently exceeded the chitin dose-dependent range, which now becomes harmful to the fish especially due to its low protein apparent digestibility (ADC) reducing the energy obtained from each gram of pellet consumed, consequentially exhibiting slower growth, lowering the SGR and also effecting the rate. Since the digestion of chitin is mainly determined by the chitobiase enzyme, where-by, higher levels of chitobiase designates greater chitin breakdown theoretically into the nutritive NAG (Fines and Holt, 2010). Different fish species have different levels of chitobiase levels, therefore limiting the ability to digest chitin to a certain range. Which in the case of marble goby, its chitobiase levels are still not specified due to incredible lack in research on the topic.

Oxyeleotris marmorata, carnivorous in the wild and tend to feed on plankton which may have given it the edge in digesting and utilising chitin to a significant yet restrictive levels (Tippayadara). Theoretically, carnivorous fish needs copious amounts of protein source, an approximate bare minimum of around 45% (Müller, Wolf and Gutzzeit, 2017). All of the formulated feeds contained protein content higher than the minimum protein required by the juvenile *Oxyeleotris marmorata*.

Chitobiase level might be one of the possible reason explaining the survival rate where the juveniles with lower chitobiase level died off being

unable digest the faecal protein, causing sink in energy. Other likely causes include problem with pellet ingestion and also the stubborn nature of *Oxyeleotris marmorata* to accept the formulated feed which is widely reported by Darwis, et al., (2008).

From the finding, it is clear that the inclusion of *Hermetia illucens* pre-pupae meal in the diet meal had significantly higher growth performance over the 12 week feeding experiment, in term of weight gain for *Hermetia illucens* pre-pupae meal content of 4% and 8% (17.03% and 25.72%, respectively) meanwhile, HM120 diet meal with 12% *Hermetia illucens* content and lower (1.55% weight gain) a and also slower (0.02 SGR) growth rate and yet still slightly higher than the meal with *Hermetia illucens* pre-pupae meal content of 0% (HM0) having gained only 1.01 g in the span of 12 week.

The increment in *Hermetia illucens* pre-pupae meal in the dietary meal lead to a boost in protein efficiency ratio for each meal, HM0 and HM120 had a PER value of 0.042 and 0.011, which indicates the lower nutritive value of the protein sources. Contrarily, the HM40 meal had PER value of 0.088, while diet meal HM80 had PER value of 0.137 indicating a better absorption and utilization of protein source by the fish.

The lipid utilisation from all previous studies suggests no negative effect where-by, the lipid utililization does not differ much with increase in *Hermetia*

illucens pre-pupae meal in the diets (Dumas, et al., 2018). Same findings are reported in the study.

Lastly, linoleic acid and α-linoleic acid were the only PUFAs produced from *Hermetia illucens* larvae and pre-pupae in the present study. The formation of C18:2n6(c) can be described and linked to the activity of *Δ12 fat 2 desaturase*, which able to convert C18:1n9 into C18:2n6(c) (Watts and Browse, 2002). The *Δ12 fat 2 desaturase* can further catalysed Δ15 desaturation C18:1n9 bio-accumulated to produce C18:3n3 (Zhou, Green and Singh, 2011). Same result was obtained in the study conducted by Ewald, et al. (2020) larvae reared with bread. Therefore, the hypothesis is drawn whereby the *Hermetia illucens* pre-pupae can be used as fish meal and fish oil replacement as it contained similar nutrient values, better compared to it larval stage.

5.2 Effect of Dietary Inclusion of *Hermetia illucens* Pre-pupae Meal on the Growth Performance and Somatic Indices of *Oxyeleotris marmorata*

Growth performance is the study of input (feed) apply on specify species (eg: fish) based on certain parameters such as weight gained and length gained after a period of time (Camargo, Aranha and Menezes, 2018). It is very importance to study growth performance for the understanding of physiological and anatomical mechanism underneath (Pauly, 1987).

In the present study, four experimental isolipid and isoproteic diets containing 10% crude lipid and 46% crude protein were investigated; Yong, Ooi and Shapawi (2013) conducted a pioneer research on *Oxyeleotris marmorata* fingerlings, where the experimental diet consisted of 10% dietary lipid and 45% dietary protein, which is almost similar to current study. Moreover, in another research by Ti, Ong and Teoh (2019) who used similar dietary lipid (12%) and dietary protein (45%) to feed *Oxyeleotris marmorata* juveniles of estimated 13.6 g in weight reported no effects on the fish whole body and fillet biochemical composition. A study by Yong, et al. (2015) found that 10% of dietary level showed the highest growth performance and feed utilization in *Oxyeleotris marmorata*. Despite this, the dietary lipid level higher than 10% did not improve the growth and feed conversion ratio. However, it directly increased the whole body weight in term of hepatic lipid and other somatic indices. When the dietary lipid level increased to 22%, the hepatosomatic index (HSI) was 4.26%, whereas viscerosomatic index (VSI) was 9.98% compared to control diet (dietary lipid level at 10%), HSI was 3.0%, whereas VSI was 6.39% (Yong, et al., 2015). The dietary lipid level higher than optimal dietary lipid level for fish requirement will induce excessive fat accumulation and resulted in higher mortality rate caused by fatty liver disease (Bundit, 2007).

In this study, all the experimental diets had a significantly different ($P<0.05$) crude lipid and crude protein content. The *Oxyeleotris marmorata* juveniles fed with diet HM80 had better weight gained, specific growth rate, feed conversion rate and protein efficiency ratio compared to other juveniles on

the experimental diets. However, juveniles fed with diet HM0 (without pre-pupae meal) showed highest survival rate, 80% compared to others. Similar result was reported by Yong, Ooi and Shapawi (2013) *Oxyeleotris marmorata* juveniles fed with control diet (without soybean meal substituted) showed 88.3% of survival rate.

From the results in Table 4.7, the HSI and VSI of *Oxyeleotris marmorata* juveniles fed on experimental diets was ranged 3.07 – 6.85% and 8.59 – 12.51%, respectively. As reported by Yong, et al. (2015) HSI was ranged 3.06 – 4.26% and VSI was ranged 6.39 – 9.98%. Notably, the HSI and VSI reported by Ti, Ong and Teoh (2019) were extremely low compared to this study.

The result of biochemical composition of whole body and fillet of *Oxyeleotris marmorata* in the present study is shown in Table 4.8. The crude lipid, crude protein and ash content in whole body composition (dry basis) was ranged from 3.46 – 6.38%, 76.1 – 79.92% and 16.94 – 18.48%, respectively. However, the crude lipid and ash content in whole body of *Oxyeleotris marmorata* juveniles reported by Ti, Ong and Teoh (2019) was ranged from 10.4 – 14.2% and 18.9 – 20.5%, respectively, which were higher values compared to current study. On the other hand, the results reported by Yong, et al. (2015) was lower compared to present study, whereby, the crude lipid was 1.53 – 3.74% and crude ash was 10.37 – 12.60%. Surprisingly, the crude protein of juveniles' whole body was higher compared to 45.58 – 53.01% in Yong, et al. (2015) and 68.0 – 71.0% in Ti, Ong and Teoh (2019). The crude lipid and crude protein

content was 0.8% and 17.4%, respectively, in juveniles' fed on pellet pH 2.5. On the other hand, the fillet composition (wet basis) of *Oxyeleotris marmorata* juveniles in present study was similar to the results reported by Ti, Ong and Teoh (2019) the crude lipid, crude protein and ash content of the fillet was 1.1 – 1.7%, 18.5 – 19.0% and 1.4 – 1.8%, respectively.

5.3 Effect of Dietary Inclusion of *Hermetia illucens* Pre-pupae Meal on the Whole Body and Fillet Composition of *Oxyeleotris marmorata*

The fatty acid profiling of whole body, fillet and liver of the experimental targeted fish species, *Oxyeleotris marmorata* were proportional to its respective dietary fatty acid composition. This undoubtedly reflected the *Oxyeleotris marmorata* juvenile ingested the dietary fatty acids from *Hermetia illucens* pre-pupae meal, fish oil and fish meal. Juveniles fed with HM120 showed higher of total MUFAs in the fillet despite of lower dietary MUFAs. The total of PUFAs (25.90 – 28.77%) found in the fillets of juvenile were relatively higher compared to dietary PUFAs (6.12 – 19.74%). Moreover, the linoleic acid (LA) rich diets especially HM0 (10.78 ± 0.38%) and HM40 (8.26 ± 0.16%) did not affect the arachidonic acid (ARA) content in the whole body, fillet and liver of juvenile. No remarkable changes were observed in the ARA level of the whole body, fillet and liver for all diet treatments. Conversely, moderately range of ARA were detected in juvenile's whole body (2.98 – 5.30%) and fillet (4.57 – 5.76%), it might due to higher initial ARA deposited in the initial stage of

juvenile's whole body (4.77 ± 0.07%) and fillet (5.45 ± 0.05%). This can be explained by the limitation of LA elongation and Δ6 desaturation in the *Oxyeleotris marmorata* juveniles as the trace amount of gamma-linoleic acid (18:3n6, 0.14 – 0.37%) and dihomo-γ–linolenic acid (20:3n6, 0.26 – 1.24%) present in the juvenile's whole body, fillet and liver. Ti, Ong and Teoh (2019) observed that *Oxyeleotris marmorata* juveniles fed with higher degree of LA diets possessed limited or no capability in bio-converting LA to ARA. In contrast, Bundit (2007) perceived *Oxyeleotris marmorata* juveniles have an inherent ability as majority freshwater fish do. Turchini et al. (2006) indicated freshwater fish have higher conversion of LA to ARA when feeding diet consisted with low levels of ARA but high levels of LA. Nevertheless, there are no many findings on fatty acid composition of *Oxyeleotris marmorata* liver and other body parts. Nonetheless, the high amount of ARA in the initial stage of *Oxyeleotris marmorata* juvenile was comparable to snakehead (*Channa micropeltes*), a carnivorous freshwater fish with similar amount of ARA content (4.71 ± 0.95%) found in Malaysia (Zuraini et al., 2006). From the same family species of snakehead (*Channa striata*), the activities of Δ4 and Δ5 desaturation were observed at 0.2% of conversion rate for desaturation efficiency of the C20:3n-6 to ARA (Kuah, et al., 2015). The natural food resources for snakehead such as shrimp, aquatic insects and fish prey with high ARA composition from it living environment explained the limitation of the activity of Δ4 and Δ5 desaturation (Carole, et al., 2005; Bundit, 2007). Taking into the consideration above, the bio-transformation or accumulation of fatty acid diversified rely on body part and metabolic aspects such as fatty acids metabolism, preferential assimilation, β-oxidation (Turchini and Francis, 2009; Teoh and Ng, 2016).

High ARA content was found in experimental *Oxyeleotris marmorata* juvenile fillet which ranged from 4.57 to 5.76% than whole body (2.98 – 5.30%) and liver (0.27 – 0.39%). Higher retention of ARA in fillet muscle explained to carry out some functional purposes such as fertility development, egg sacs quality and higher larvae survival rate at initial stage as observed in *Paralichthys olivaceus* (Japanese flounde), *hippoglossus* (Atlantic halibut), *Gadus morhua* (Atlantic cod). (Bell and Sargent, 2003; Furuita et al., 2003; Mazorra et al., 2003; Røjbek et al., 2012. Additionally, ARA can further improved pressure anxiety acceptance and immunity in *Siganus rivulatus* (rabbitfish) and *Sparus aurata L.* (gilthead seabream). These findings revealed that the understanding of *Oxyeleotris marmorata*, fish that has slightly extraordinary stress tolerance and reproductivity. On the other hand, n-3 PUFAs in entire fish body, fillet and liver mirrored intently that of trial diets and this showed that dietary n-3 unsaturated fatty acid could be ingested and retained in the fish.

CHAPTER 6

CONCLUSION

Based on the results obtained, the crude lipid and crude protein content of *Hermetia illucens* pre-pupae was 46.83 ± 1.37% and 43.84 ± 1.08%, respectively. The physicochemical properties of *Hermetia illucens* pre-pupae lipid such acid value (2.59 ± 0.58 mgKOH/g), iodine value (23.76 ± 0.34 gI_2/100g) and peroxide value (1.11 ± 0.21 mEq/kg), which met the FAO standard and safe to use as fish oil replacement. Moreover the FA class of pre-pupae dominated by ΣSFA (78.18 ± 1.73%), followed by ΣMUFA (14.53 ± .21%) and the least was ΣPUFA (7.28 ± 0.57%).

Additionally, the formulated diets with different inclusion levels of *Hermetia illucens* pre-pupae meal contained 9.57 – 10.26% of crude lipid, 45.06 – 47.98% crude protein and 15.49 – 16.10 MJ/kg of energy which fulfilled the basic nutrients level required for the growth of *Oxyeleotris marmorata* juveniles. After 12 weeks of experimental feeding trial, the weight gained, specific growth rate, feed conversion rate, protein efficiency and survival rate of the juveniles obtained were 1.01% to 25.72%, 0.02 g day^{-1} to 0.27 g day^{-1}, 15.27 g feed/ g weight gained to 188.52 g feed/ g weight gained, 0.011 g weight gained/ g protein to 0.137 g weight gained/ g protein and 40% to 80%, respectively.

The research findings indicated that HM40 was the best *Hermetia illucens* diet as it induces the second best growth performace while having the highest survival rate of 60% of *Oxyeleotris marmorata*. Although HM80 diet prompts the greatest growth in *Oxyeleotris marmorata,* however, since the diet has seemingly exceeded the chitin dose-dependent range, the survival rate is reduced ominously low to just a bare 40%. Thus, diet with 4% inclusion of *Hermetia illucens* is the best diet for *Oxyeleotris marmorata*, both survival and growth wise.

For the biological indices, the fillet yield of juveniles was 36.68% – 37.36%. However, the vicerosomatic and hepatosomatic index was 8.59% – 12.51% and 3.07% – 6.85%, respectively. Lastly, the fatty acid profile of *Oxyeleotris marmorata* juvenile's fillet was dominated by ΣSFA (44.88 – 52.94%) followed by ΣPUFA (25.90 – 28.77%) and ΣMUFA (21.16 – 26.35%).

In overall, the *Oxyeleotris marmorata* fed with diet HM40 (4% inclusion level of *Hermetia illucens* pre-pupae meal) showed the best growth performance while having the highest survival rate when compared to other diets. The juvenile gained 17.03% of weight and showed 60% of survival rate. Furthermore, the fatty acid profile of juvenile fed with HM40 retained 49.06 ± 0.07% of ΣSFA, followed by 27.35 ± 0.12% of ΣPUFA and 23.59 ± 0.09% of ΣMUFA.

Future work should be more focused on improving the digestibility of the feed, by using defatted *Hermetia illucens* in order to increase the survival rate of the *Oxyeleotris marmorata*. Other than that, feeding trials of the *Hermetia illucens* diet should tried on other fishes with higher inclusions. Beasides that, study on the feeding trials of other insects besides *Hermetia illucens* as fish meal replacement should be carried out on *Oxyeleotris marmorata.*

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APPENDICES

Appendix A: Proximate Analysis

Table A1: Moisture Content of *Hermetia illucens* Larvae and Pre-pupae Meals and Fish Meal

Sample	Weight of fresh sample (g)			Weight of dried sample (g)			Moisture content (g)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Larvae meal	15.7695	15.1971	15.5299	5.75508	5.54990	5.65662	63.53	63.49	63.58	63.53	0.04
Pre-pupae meal	10.5315	10.3773	10.4683	3.94428	3.83330	3.93428	62.56	63.06	62.43	62.69	0.27
Fish meal	10.4253	10.2561	10.2891	10.2648	10.1038	10.1311	1.54	1.48	1.54	1.52	0.02

Calculation:

$$\text{Moisture content, \%} = \frac{\text{Weight of fresh sample (g)} - \text{Weight of dried sample (g)}}{\text{Weight of fresh samples (g)}} \times 100\ \%$$

$$= \frac{15.7695\ \text{g} - 5.7508\ \text{g}}{15.7695\ \text{g}} \times 100\ \%$$

$$= 63.53\ \%$$

Table A2: Crude Lipid Content of *Hermetia illucens* Larvae and Pre-pupae Meals and Fish Meal

Sample	Weight of sample (g)			Weight of lipid extracted (g)			Lipid content (%)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD

Larvae meal	10.0356	10.0338	10.0204	4.8730	4.4928	4.8932	48.56	44.78	48.83	47.39	1.85
Pre-pupae meal	10.0399	10.0258	10.0507	4.8943	4.6256	4.5832	48.75	46.14	45.60	46.83	1.37
Fish meal	9.9834	10.0903	10.0448	1.5702	1.5691	1.5554	5.73	15.55	15.48	15.59	0.10

Calculation:

$$\text{Crude lipid content, \%} = \frac{\text{Weight of lipid extracted (g)}}{\text{Weight of sample (g)}} \times 100\ \%$$

$$= \frac{4.8730\ \text{g}}{10.0356\ \text{g}} \times 100\ \%$$

$$= 48.56\ \%$$

Table A3: Crude Protein Content of *Hermetia illucens* Larvae and Pre-pupae Meals and Fish Meal

Sample	Weight of defatted sample (g)			Volume of titrant used (mL)			Normality of titrant, HCl (N)			Percentage of nitrogen (% N)			Crude protein content (%), without chitin correction				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Larva meal	1.000			88.6	89.3	88.9	0.1			12.404	12.502	12.446	77.53	78.14	77.79	77.82	0.25
Pre-pupae meal				100.7	100.9	100.1				14.098	14.126	14.014	88.11	88.29	87.59	88.00	0.30
Fish meal				91.8	91.3	92.1				12.852	12.782	12.894	80.3	79.89	80.59	80.27	0.29

Calculation:

$$\text{Percentage of nitrogen, \%} = \text{Normality of HCl} \times \frac{\text{Volume of titrant used (mL)}}{\text{Weight of defatted sample (g)}} \times 14.006 \times 100$$

$$= 0.1 \times \frac{88.6\ \text{mL}}{1.000\ \text{g}} \times 14.006 \times 100\ \%$$

$$= 12.404\ \%$$

$$\text{Percentage of protein, \%} = \text{Percentage of nitrogen, \%} \times 6.25$$

$$= 12.404\ \% \times 6.25$$

= 77.53 %

Table A4: Chitin extraction of *Hermetia illucens* Larvae and Pre-pupae Meals

***Hermetia illucens* larvae**	**Weight of sample (g)**			**Weight of chitin extracted (g)**			**Percentage of chitin extracted (%)**				
	R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**AVG**	**SD**
Larvae meal	5.0872	5.0753	5.0482	0.2873	0.2460	0.3090	5.06	4.85	6.12	5.34	0.56
Pre-pupae meal	5.0353	5.0715	5.0694	0.5050	0.7295	0.5015	10.03	14.38	9.89	11.44	2.09

Calculation:

$$\text{Percentage of chitin extracted, \%} = \frac{\text{Weight of chitin extracted (g)}}{\text{Weight of sample (g)}} \times 100\ \%$$

$$= \frac{0.2873\ \text{g}}{5.0872} \times 100\ \%$$

$$= 5.06\ \%$$

Table A5: Ash Content of *Hermetia illucens* Larvae and Pre-pupae Meals and Fish Meal

Sample	Weight of defatted sample (g)			Weight of ash (g)			Ash content (%)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Larvae meal	1.0707	1.0683	1.0659	0.1533	0.1634	0.1630	14.32	15.30	15.29	14.97	0.46
Pre-pupae meal	1.0577	1.0892	1.0727	0.0984	0.1362	0.1098	9.30	12.50	10.24	10.68	1.34
Fish meal	1.0292	1.0709	1.0764	0.2207	0.2295	0.2303	21.44	21.43	21.40	21.42	0.02

Calculation:

$$\text{Ash content, \%} = \frac{\text{Weight of ash (g)}}{\text{Weight of defatted sample (g)}} \times 100\ \%$$

$$= \frac{0.1533\ \text{g}}{1.0707\ \text{g}} \times 100\ \%$$

$$= 14.32\ \%$$

Table A6: Gross Energy of *Hermetia illucens* Larvae and Pre-pupae Meals and Fish Meal

Samples	Gross energy (MJ/ kg)				
	R1	R2	R3	AVG	SD
Larvae meal	27670.8	26601.2	27333.1	27201.70	444.4
Pre-pupae meal	27966.4	27808.4	27700.8	27825.20	109.1
Fish meal	20356.9	20347.3	20271.3	20325.17	38.29

Appendix B: Lipid Properties of *Hermetia illucens* Larvae

Table B1: Peroxide Value of Larvae Meal and Pre-pupae Meal for *Hermetia illucens* Larvae

Hermetia illucens larvae	Weight of the sample (g)			Volume of titrant, BLANK (mL)			Volume of titrant, SAMPLE (mL)			Normality of $Na_2S_2O_3$ (N)			Iodine value (g I_2/ 100 g)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Larvae meal	1.0333	1.0157	0.0135	0			0.10	0.15	0.10	0.01			0.97	1.48	0.99	1.14	0.24
Pre-pupae meal	1.0525	1.0646	1.0253				0.10	0.15	0.10				0.95	1.41	0.98	1.11	0.21

Table B2: Iodine Value of Larvae Meal and Pre-pupae Meal for *Hermetia illucens* Larvae

Hermetia illucens larvae	Weight of the sample (g)			Volume of titrant, BLANK (mL)			Volume of titrant, SAMPLE (mL)			Normality of $Na_2S_2O_3$ (N)			Iodine value (g I_2/ 100 g)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Larvae meal	0.11770	0.12442	0.11225	36.1			34.0	34.9	34.7	0.1			22.78	12.26	15.79	16.94	4.37

Pre-pupae meal	0.1188	0.1099	0.1186		33.9	34.0	33.9		23.50	24.25	23.54	23.76	0.34

Table B3: Acid Value of Larvae Meal and Pre-pupae Meal for *Hermetia illucens* Larvae

***Hermetia illucens* larvae**	**Weight of the sample (g)**			**Volume of titrant, BLANK (mL)**			**Volume of titrant, SAMPLE (mL)**			**Normality of KOH (N)**			**Acid value (mg KOH/ g)**				
	R1	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**R1**	**R2**	**R3**	**AVG**	**SD**
Larvae meal	0.9632	1.0469	1.0095	2.7			66.6	66.1	66.2	0.01			2.27	1.82	1.95	2.01	0.19
Pre-pupae meal	1.0235	1.0417	1.0321				88.9	66.5	77.0				3.40	2.05	2.34	2.59	0.58

Appendix C: Growth Performances of *Oxyeleotris marmorata* Juveniles

Table C1: Parameters of *Oxyeleotris marmorata* recorded

Parameters	Experimental diets			
	HM0	**HM40**	**HM80**	**HM120**
Initial weight (g)	15.61	18.09	15.94	16.16
Final weight (g)	16.62	21.17	20.04	16.41
Total feed consumption (g)	51.72	74.29	62.60	47.13
Crude protein* (%)	45.06	46.94	47.55	47.98

*crude protein content with chitin correction.

Calculation:

Specific growth rate based on weight gained (SGR_W), % growth/ day,

$$SGRw = \frac{\ln W_t - \ln W_0}{t} \times 100\ \%$$

$$= \frac{\ln 16.62 - \ln 15.61}{12\ \text{week} \times 7\ \text{days}} \times 100\ \%$$

$$= 0.07\ \%\ \text{of growth per day}$$

Feed conversion rate (FCR),

$$FCR = \frac{\text{Total feed consumed (g)}}{\text{Weight of fish gained (g)}}$$

$$= \frac{51.72\ \text{g}}{16.62\ \text{g} - 15.61\ \text{g}}$$

$$= 51.21$$

Protein efficiency ratio (PER),

$$PER = \frac{\text{weight of fish gained (g)}}{\text{protein intake (g)}}$$

$$= \frac{16.62\ \text{g} - 15.61\ \text{g}}{51.72\ \text{g} \times 45.06\ \%}$$

$$= 0.043$$

Table C2: Survival Rate of *Oxyeoleotris marmorata* Jevuniles Fed with Different Experimental Diets

Number of fishes survived (week)												
Week / Diet	1	2	3	4	5	6	7	8	9	10	11	12
HM0	10	10	10	10	10	10	10	9	9	9	8	8
HM40	10	10	10	10	9	9	9	9	7	6	6	6
HM80	10	10	10	10	10	10	10	9	7	5	5	4
HM120	10	10	10	10	10	10	10	9	8	7	7	5

Calculation:

$$\text{Survival rate} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100\ \%$$

$$= \frac{8}{10} \times 100\ \%$$

$$= 80\ \%$$

Table C3: Fillet Yield of *Oxyeoleotris marmorata* Jevuniles Fed with Different Experimental Diets

Diet	Weight of whole fish (g)			Weight of fillet (g)			Fillet yield (%)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Initial	9.84	13.40	14.30	3.33	5.13	5.16	33.84	38.28	36.08	36.07	1.81
HM0	13.97	19.99	22.34	4.58	8.17	8.38	32.78	40.87	37.51	37.06	3.32
HM40	23.48	21.90	28.83	7.61	9.00	11.12	32.41	41.10	38.57	37.36	3.65

HM80	24.70	22.18	18.72	9.39	8.14	6.61	38.02	36.70	35.31	36.68	1.11
HM120	17.72	19.90	15.90	6.06	7.73	6.25	34.20	38.84	39.31	37.45	2.31

Calculation:

$$\text{Fillet yield, \%} = \frac{\text{weight of fillet (g)}}{\text{weight of whole fish (g)}} \times 100\ \%$$

$$= \frac{3.33}{9.84} \times 100\ \%$$

$$= 33.84\ \%$$

Table C4: Viscerosomatic Index of *Oxyeoleotris marmorata* Jevuniles Fed with Different Experimental Diets

Diets	Weight of whole fish (g)			Weight of Viscera (g)			Viscerosomatic index (%)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Initial	9.84	13.40	14.30	1.25	1.17	1.43	12.70	8.73	10.00	10.48	1.66
HM0	13.97	19.99	22.34	1.13	2.33	1.90	8.09	11.66	8.50	9.42	1.59
HM40	23.48	21.90	28.83	2.85	2.09	3.04	12.14	9.54	10.54	10.74	1.07
HM80	24.70	22.18	18.72	3.14	2.58	2.47	12.71	11.63	13.19	12.51	0.65
HM120	17.72	19.90	15.90	1.35	1.91	1.36	7.62	9.60	8.55	8.59	0.81

Calculation:

$$\text{Viscerosomatic index (VSI), \%} = \frac{\text{weight of viscera (g)}}{\text{weight of whole fish (g)}} \times 100\ \%$$

$$= \frac{1.25}{9.84} \times 100\ \%$$

$$= 12.70\ \%$$

Table C5: Hepatosomatic Index of *Oxyeoleotris marmorata* Jevuniles Fed with Different Experimental Diets

Diets	Weight of whole fish (g)			Weight of Liver (g)			Hepatosomatic index (%)				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	AVG	SD
Initial	9.84	13.40	14.30	0.38	0.35	0.48	3.86	2.61	3.36	3.28	0.51
HM0	13.97	19.99	22.34	0.36	0.94	0.77	2.58	4.70	3.45	3.58	0.87
HM40	23.48	21.90	28.83	1.07	0.75	1.35	4.56	3.42	4.68	4.22	0.57
HM80	24.70	22.18	18.72	1.80	1.45	1.26	7.29	6.54	6.73	6.85	0.32
HM120	17.72	19.90	15.90	0.39	0.83	0.45	2.20	4.17	2.83	3.07	0.82

Calculation:

$$\text{Hepatosomatic index (HSI), \%} = \frac{\text{weight of liver (g)}}{\text{weight of whole fish (g)}} \times 100\ \%$$

$$= \frac{0.38}{9.84} \times 100\ \%$$

$$= 3.86\ \%$$

LIST OF PUBLICATIONS

Chin, Y. C., Leong, S. Y., Lo, P. K., Tey, L. H. and Loo, J. L., 2019. Comparison of *Hermetia illucens* larvae and pre-pupae as potential aqua feed derived from the biotransformation of organic waste. *AIP Conference Proceedings*, 2157, 20008.

CONFERENCE ATTENDED

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