**Faculty of Engineering and Sciences**

# Physiological changes of post-puerulii spiny lobster (*Panulirus ornatus* Fabricius, 1798) fed low protein to energy ratio diets supplemented with black soldier fly (*Hermetia illucens*) meal

**Ishaaq Saputra**
**0000-0001-9056-2151**

**This thesis is presented for the Degree of**

**Doctor of Philosophy**

**of**

**Curtin University**

**July 2024**

**DECLARATION**

To the best of my knowledge and belief, this thesis contains no material previously published by any other person except where due acknowledgment has been made. This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Animal Ethics: Animal ethics approval from the proposed research study was not required as it is not mandatory for the invertebrate animal studies at Curtin University, Malaysia. However, as the research was conducted in Indonesia, the required protocols were followed while handling the animals, as per the guidelines of Animal Welfare Act No.18 2009 of the Republic of Indonesia.

Signature 

Date: July 2024

This thesis is dedicated to my parents, two significant people in my life, who taught me to learn, work hard and persist to achieve success. My beloved wife, Nurleli, spent her time, energy, and patience on my education goal, so I could continue my academic journey. My kids, Raveena Nurlaksita, Dhiera Nurasvanenggala, and Giandranaya Prahasta and their everyday support kept me stronger and sane during the time of the study. I am extremely grateful for their constant support, and encouragement and for never giving up on me.

**ACKNOWLEDGEMENTS**

I would like to express my gratitude to Curtin University Malaysia for supporting my PhD journey through the Curtin Malaysia Postgraduates Studentship Scholarship. I want to seize this opportunity to extend my appreciation to Professor Ravi Fotedar, who not only provided me with the chance to pursue my academic aspirations but also served as a constructive critic, engaging in healthy discussions and offering valuable criticism. Professor Ravi, I am truly thankful for your unwavering support and encouragement, starting from the challenging admission process through to my entire PhD journey. Your wisdom and guidance have been instrumental in helping me navigate cultural differences and adapt to the various systems in Malaysia. I am also grateful for the assistance you provided during my master's program, which eventually led to the scholarship enabling me to continue my academic pursuits. A heartfelt thank you goes to my co-supervisor, Professor Beena Giridharan, and Dr. Yih Nin Lee for their guidance and support whenever I faced challenges. Professor Beena Giridharan's significant contributions were particularly valuable in planning experiments, writing manuscripts, and navigating the admission process at Curtin Malaysia. Thank you for Dr. Yih Nin Lee for her guidance at the end of my study, organizing internal meeting, and supporting my study completion. A special thanks to my beloved wife Nurleli, for supporting me during the experiment. I appreciate that she has helped me to go through tough times during my Ph.D. Huge gratitude to Dr. Bayu Priyambodo, local advisor for my study for giving me a valuable discussion and sharing knowledge about lobster aquaculture in Indonesia. His suggestions and advice make my point of view about lobster wider. Thanks to Dr. Putu Eka Sudaryatma for his unique technical assistance during the laboratory work, especially in gene expression analysis. Special thanks to Dr. Dodi Hermawan for supporting me in getting feed ingredients during my experiment and sample analysis. Thanks to the Fish Quarantine and Inspection Agency Jakarta I Laboratory for giving me the opportunity to conduct my gene expression samples analysis.

## TABLE OF CONTENTS

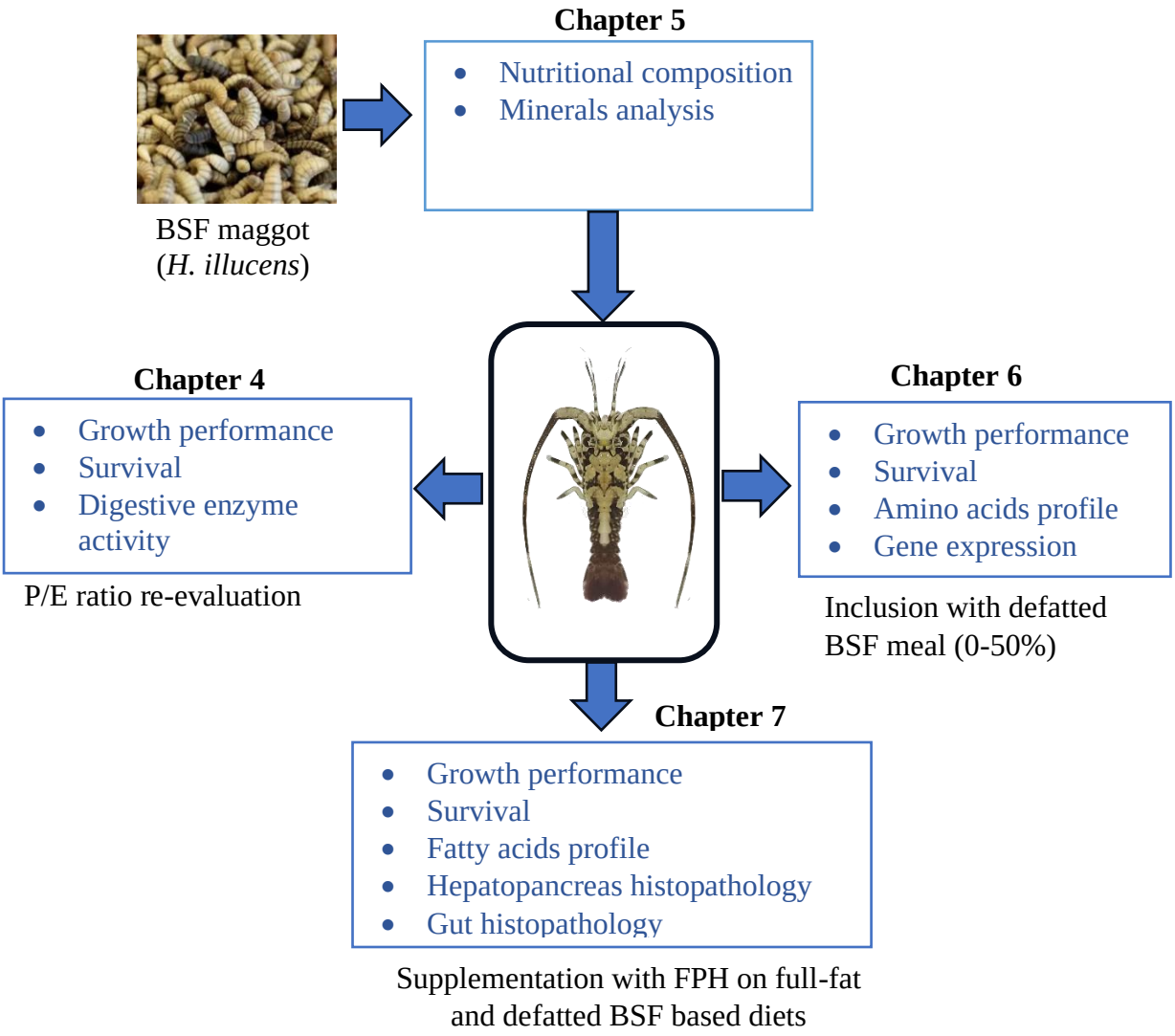
**LIST OF TABLES**

## LIST OF FIGURES

## PREAMBLE

This research aimed to evaluate the effects of a low protein to energy ratios of black soldier fly meal-supplemented diets with black soldier fly (BSF) (*Hermetia illucens*) meal and fish protein hydrolysate (FPH) on the growth, survival, and immune response of post-puerulii ornate spiny lobster (*Panulirus ornatus*). The thesis consists of eight chapters. Chapter 1, the introduction, provides an overview of the thesis with a brief description of spiny lobster production, both from wild-caught and aquaculture globally and the importance of spiny lobsters in the aquaculture industry of Indonesia. Chapter 2 reviews the published literature on the biology, lifecycle, and production of spiny lobster. The review also focuses on the importance of nutrition research in tropical spiny lobsters. It also covers the importance of fishmeal replacement in the crustaceans' growth, survival, health indices and diet. Chapter 3 presents a general methodology used during the research. It explains the materials and methods common to all research based chapters. It also covers the general details of the experimental setup. Chapter 4 (published in "Annals of Animal Science") re-evaluates of the P/E ratio of formulated diets for juvenile lobster and their effects to the growth, survival digestive enzyme activity, proximate body composition and amino acids. Chapter 5 (Published in "Biodiversitas") evaluates the nutritional composition of full fat and defatted BSF. Chapter 6 (Published in "Aquaculture International") evaluates the effects of defatted BSF meal inclusion on the physiology, hepatopancreas histology and immunology-relatives gene of juvenile lobster. Chapter 7 (Published in "Aquaculture Nutrition") describes the effects of supplementation of defatted, full fat black soldier fly and fish protein hydrolysate on the physiology and immunology-relatives gene of juvenile lobster. Chapter 8 Discusses the findings of all chapters and presents the conclusions, limitation of the present study and recommendations for the future research.

**GRAPHICAL ABSTRACT**



**ABSTRACT**

The ornate spiny lobster (*Panulirus ornatus*) has a high commercial value as one of several known marine rock lobsters and has been studied for its potential for aquaculture. Among several constraints on spiny lobster aquaculture, nutrition of the diet for spiny lobster has become a major topic for researches. Although there are some studies on the aquadiet for spiny lobsters, the suitable protein to energy (P/E) ratio on the feed for optimum growth and immunology condition is poorly understood. In addition, there is no information available when the diets have a minimum or no fishmeal in them. The first trial was to re-investigate the effect of various P/E ratios on the growth, digestive enzyme activities and body compositions of the spiny lobster juvenile. The results showed that the optimum growth, survival and digestive enzyme activities of juvenile lobsters were achieved at the P/E ratio of 26.6 g CP $MJ^{-1}$. The second and third trial objectives were to analyse the nutritional contents of black soldier fly (*H. illucens*) meal and their effect on the chemical and physical properties as protein sources in the diet for *P. ornatus.* Results indicated that the defatted BSFL have higher crude protein content than full fat BSF, with the crude protein value at 47.70% and 30.72%, respectively. The full fat and defatted BSF larvae meal have comparable nutrition value to fishmeal and the defatting process of BSFL improves the nutrition content of BSFL including protein and amino acids. The third trial was conducted to evaluate the optimum inclusion level of defatted black soldier fly meals inclusion on the physiology, hepatopancreas histology and the expression immunology-relatives gene of juvenile lobsters (*P. ornatus*). The results showed that fishmeal is technically a suitable aquafeed ingredient in terms of crude protein, moisture, and amino acid compositions. At the end of week 8 of the feeding trial, the specific growth rate, length increment, survival rate, and moulting rate of juvenile lobster were the same. The cytokine cell expression analysis indicated that up to 35% of fishmeal replacement increased the inflammatory cytokine cells (Interleukin 8 and 17) of the juvenile lobsters, while an adverse impact was observed on juvenile lobsters receiving 50% of fishmeal replacement by BSF meal.

Histopathological analysis showed that the hepatopancreas cells of juvenile lobsters were damaged following fishmeal replacement beyond 35%. The fourth trial was performed to evaluate the effect of fish protein hydrolysate supplementation in the experimental diets containing full fat and defatted BSF meal on the growth, survival, histopathology, and body composition of juvenile lobsters (*P. ornatus*). The results indicated that the specific growth rate, final body weight, final total length, and length increment of the juvenile lobster were significantly affected by the fishmeal substitution and the addition of liquid FPH in the feeds containing full-fat and defatted BSF improved those physiological parameters. The size of villus and muscle thickness in the digestive tract was the same following FPH supplementation. In addition, the hepatopancreas histopathology indicated the presence of B-cells and R-cells in the juvenile lobster fed with FPH-supplemented feeds. In summary, the supplementation of liquid FPH in a low P/E ratio diet with BSF inclusion could improve the physiological and immunological response of juvenile lobster. Further validation of the present results in required at a commercial and/or pilot scale.

# CHAPTER 1: INTRODUCTION

## 1.1. Background

Lobster fisheries have become lucrative in the global fishing industry with a total production of 231,968 tonnes in 2013. The number was doubled compared to the total capture in 1980. Among the total landings of marine lobster, the spiny lobster (Panulirus spp.) accounted for 75,953 tonnes or 32.74%. The American lobster (*Homarus americanus*) has a production proportion of 60% of the total landings, while tropical spiny lobster (*P. argus, P. ornatus* and *P. homarus*) production accounted for only 13.9 % (FAO 2017). As the wild-caught production stagnated, the aquaculture of spiny lobster became a sustainable approach to fulfilling the increasing demand for this luxurious crustacean commodity (Phillips and Matsuda 2011; Radhakrishnan et al. 2020). Although the aquaculture of spiny lobsters began years ago, the development of lobster aquaculture was too slow due to the complexity of the culture system including seed production in hatcheries, feeding and other technological issues. The major countries producing spiny lobsters are Australia, India, Vietnam and New Zealand while several other countries have small portions of global lobster production (Phillips and Matsuda 2011). Among these nations, Vietnam is the only country that has a commercial scale of spiny lobsters, with the total production of spiny lobsters from aquaculture reaching 1,900 tonnes in 2006 (Jones 2009b). Due to a disease problem in 2007-2009, the Vietnamese spiny lobster aquaculture production decreased by 50% before it remained stable in the years after with an average annual harvest of 1,500 tonnes of spiny lobsters (Anh and Jones 2015). The spiny lobster aquaculture is a promising industry in the present and future. However, considerable effort is still needed for further development, particularly in the nutritional area. Although some of the research on the nutritional status and requirements of the spiny lobster has been established, little is known about the study of protein to energy (P/E) ratio from various non-fishmeal protein-formulated diets in the ornate spiny lobster.

To date, the development of spiny lobsters has shifted from wild collection to aquaculture-based production. Therefore, understanding the nutritional requirements and the development of a pelleted diet or complete diet of this crustacean species becomes vital and requires future research (Phillips and Matsuda 2011; Francis et al. 2014; Perera and Simon 2014). Parallel with that the attempts on the use of various natural diets as part of fishmeal replacement (Smith et al. 2003; Smith et al. 2005; Cox and Davis 2009), optimum protein and lipid requirements (Glencross et al. 2001; Smith et al. 2005; Irvin and Williams 2006; Rathinam et al. 2009), amino acid requirements, (Ward 2005; Johnston et al. 2008; Jones 2010) and diet supplements (Irvin and Shanks 2015) for spiny lobsters were explored.

Nutritional studies of protein requirement using a pelleted diet for *P. ornatus* have been reported (Smith et al. 2003) and optimized the following year (Smith et al. 2005). These studies later became the main references for the nutritional experiment in most spiny lobster species including *P. ornatus* (Williams 2004; Irvin and Williams 2006; Johnston 2006; Irvin and Williams 2007; Johnston et al. 2008), *P. homarus* (Jeyakumar et al. 2004; Rathinam et al. 2009; Ridwanudin et al. 2018; Goncalves et al. 2020), *P. argus* (Rodríguez-Viera and Perera 2012; Rodríguez-Viera et al. 2017), and *Jasus edwardsii* (Ward 2005; Johnston 2006). However, those studies have not been updated until a recent work was conducted (Irvin and Shanks 2015) albeit with a limitation in the sources of the ingredients. Fishmeal was found to be a suitable ingredient in formulating diets for *P. ornatus* (Irvin and Williams 2007) and attempts of fishmeal substitution in this species using various ingredients have been reported (Smith et al. 2003; Smith et al. 2005; Irvin and Williams 2007; Johnston et al. 2008; Kurnia et al. 2017; Marchese et al. 2018). In using alternative ingredients for substituting fishmeal in the diet of the fish, several properties aspects were taken into consideration. General properties such as the digestibility of total dry matter and proximate value of protein, fat, ash, and gross energy within the ingredients are crucial factors in deciding the use of alternative ingredients. Selected fresh natural diets such as blue mussel and frozen seafood with high protein content were reported to be suitable for the spiny lobsters under experimental conditions (Smith et al. 2005;

Irvin and Williams 2006) except for squid meal. Squid meal has a consistent result in suboptimal digestibility which affects the growth of lobsters (Irvin and Williams 2007; Rodríguez-Viera and Perera 2012). Irvin and Williams (2007) also reported the digestibility of various alternative protein sources both from marine and terrestrial plants. In the animal ingredients group, krill meal and mussel flesh were superior, having higher dry matter and crude protein digestibility compared to others while lupin meal was the best plant ingredient protein resource. Irvin and Shanks (2015) reported that the using of fishmeal with unknown quality affected the biological responses of spiny juvenile lobsters. In that study, it was also reported that the inclusion of trash feed without nutrition addition affects the growth of spiny lobster juveniles negatively. According to Smith et al. (2005) an additional feed supplement such as krill meal was reported to be important in the diet formulation for juvenile lobsters vis-a-vis improving growth and survival. In addition, the supplementation not only promotes the growth of spiny lobster juveniles but also improves diet attractiveness. Up to 10% of the inclusion of krill meal as supplements in the diet of initial stages of *P. ornatus* was recommended to maintain optimum growth of spiny lobster juveniles (Marchese et al. 2018).

The evaluation of the P/E ratio in fish is critical due to their biological associations. Protein requirement was reported to have sparing energy function in the fish diet (Ai et al. 2004). Studies of the optimum P/E ratio requirement for fish had been conducted (Woolley et al. 2010; Li et al. 2012; Taynor 2013; Anizah et al. 2017; Kabir et al. 2019; Khan et al. 2019) and reviewed (Khan et al. 2019). By contrast, minimal studies were conducted on crustaceans (Goda 2008; Hu et al. 2008; Venero et al. 2008; Cui et al. 2017; Zhang et al. 2017; Hamidoghli et al. 2018; Méndez-Martínez et al. 2018). To date, the evaluation of the P/E ratio in *P. ornatus* has not been thoroughly investigated. Some research on spiny lobsters included additional pieces of information on the P/E ratio in the tested diet but not as the subject of the studies (Smith et al. 2005; Ward 2005; Johnston et al. 2008). A positive correlation between the increase in the P/E ratio with the growth performance in the lobsters has been reported (Smith et al. 2005; Huo et al. 2014). In contrast, Simon and Jeffs (2013)

reported that the different P/E ratios in the formulated diet for *J. edwardsii* did not affect the mean body weight of the lobsters. A formulated diet containing a series of carbohydrate inclusion from maize starch was set to have P/E from 15 to 34.9 g CP $MJ^{-1}$. Although the growth of lobster juveniles was not different after 3 weeks of feeding, the availability of glucose, glycogen, and soluble protein of high inclusion of maize starch was higher than others. Further, the lower energy diet (15.0 g CP $MJ^{-1}$) was more stable in terms of water immersion than higher P/E ratio diets (Simon and Jeffs 2013). Smith et al. (2005) found that a P/E ratio of 22.8 g CP $MJ^{-1}$ with 47% crude protein content is suitable for juvenile *P. ornatus* and resulted in the optimum growth. In that study, the growth of spiny lobster juveniles fed a pelletized diet was better than the juveniles fed fresh mussels with an unknown P/E ratio. The growth responses of lobster juveniles tend to improve when the P/E ratio is increased. In recent research on *J. edwardsii,* the inclusion of carbohydrates in the diet was found to be important in maintaining the dietary energy requirements for this species. For comparison, the diet for giant tiger shrimp or *P. monodon* having the protein content 33-44%, resulted on the P/E ratio of 26.65 to 34.91 g CP $MJ^{-1}$ (Chuntapa et al. 1999). In different crustacean species, two recent studies indicated that a lower optimum P/E ratio requirement was observed in *Litopenaeus setiferus* and *L. vannamei* (Guzman et al. 2001; Hu et al. 2008). The juvenile of *L. setiferus* fed with a formulated diet containing 40% crude protein and having 28.8 g CP $MJ^{-1}$ resulted in optimum weight gain. Above that level, the growth of juvenile *L. setiferus* was observed to be depressed and carbohydrates as sparing energy resources may not replace the roles of the protein in the diet formulation (Guzman et al. 2001). A study on *L. vannamei* revealed the energy requirement for optimal growth and survival was at a crude protein content of 34% and P/E ratio of 21.1 g CP $MJ^{-1}$. Despite the survival rate being similar among the treatments, maximum growth was achieved (Hu et al. 2008). The maximum growth of freshwater crustaceans *Macrobrachium nipponense* under laboratory conditions was achieved at P/E ratio of 16.49 g CP $MJ^{-1}$ with 33% of crude protein which is lower than in brackish and marine crustaceans (Zhang et al. 2017).

Of the alternative protein sources in the crustaceans' feed formulation, black soldier fly meal or BSF plays an important role (Foysal et al. 2019; Chen et al. 2021; Richardson et al. 2021; He et al. 2022). The incorporation of BSF meal in the experimental feed for fishes such as salmon (Belghit et al. 2019; Fisher et al. 2020; Weththasinghe et al. 2021), catfish (Adeoye et al. 2020), tilapia (Devic et al. 2018; Yildirim-Aksoy et al. 2020) and crustaceans (Cummins et al. 2017; Foysal et al. 2019; Saputra and Fotedar 2023) have been reported to have positive impacts. Cummins et al. (2017) reported the possibility of BSF inclusion as fishmeal substitutes in shrimp culture. In addition, Foysal et al. (2019) found that the supplementation of BSF meal improves the gut microbiota of maron (*Cherax cainii*). However, its application in spiny lobster aquaculture is not known. The use of alternative protein sources in aquafeeds was reported to affect the physical and biochemical of the feeds (Sudaryono 2001; Mohamad et al. 2021). It has been reported that physical properties such as feed stability (Ighwela et al. 2013), durability (Haetami et al. 2017) and palatability (Al-Souti et al. 2019) are the most affected quality of the feeds following fishmeal replacement.

Protein hydrolysate has been reported to improve the digestibility of several feed ingredients so that the absorption of those feed ingredients increases and gives maximum growth results. The protein hydrolysate can be used solely as a protein source or as a supplement in feed formulation. Apart from supporting the growth of the fish, the incorporation of protein hydrolysate also improved the immunity of crustaceans. In several crustacean species, the fish protein hydrolysate improves growth, survival rate and immunity. However, excessive use of protein hydrolysate was also reported. In recent years, the immune evaluation method in crustaceans is developing, taking advantage of the latest technological capabilities. With the ability of the new equipment and methods, the level of immunity of crustaceans can be analysed up to the level of gene expression with better accuracy. Haryanti et al. (2017) applied the use of gene expression to assess the immune response of scalloped lobster seeds, *P. homarus* after being given treatment in the form of adding probiotics. That study resulted in satisfactory results where the gene expression can be used to

analyse the immunological condition of lobster seeds. Additionally, Foysal et al. (2019) reported a similar method used to evaluate the effect of adding a BSF meal to the diet on the immunity of Australian crayfish, maron.

The tropical spiny lobster is one of the most important cultured species and the nutritional study on the species is still very scarce. The main references of the nutritional study only use limited alternative ingredients (Smith et al. 2003; Smith et al. 2005; Irvin and Williams 2006; Williams 2007). Research on ornate spiny lobster farming has a noticeable improvement in the last decades due to high demand for the commodity in the commercial market. However, there is still a knowledge gap in many biological aspects of this species, particularly on the nutrition requirements. Understanding the nutrition requirements of spiny lobsters is a critical pathway that will improve the knowledge and fill those gaps. Besides fishmeal substitution using both plant and animal-derived protein sources at a certain level has been reported to be acceptable. A recent update of the feeding study in *P. ornatus* was conducted only with few nutritional aspect improvements (Irvin & Shanks, 2015). In some of the crustaceans, protein to energy was found to have more effects on the growth and immunology condition although this study on the ornate spiny lobster remains unclear. Therefore, a study on the optimum P/E ratio in the diet with various inclusion levels of new alternative protein source ingredients for spiny lobsters is an important milestone in spiny lobster aquaculture. Taking into consideration the evaluation of the various level of protein to energy ratio from non-fishmeal diet for spiny lobster juvenile is important and needs to be investigated.

## 1.2. Aim

The aim of the present study is to investigate the growth and physiological responses of post- puerulii ornate spiny lobsters (*P. ornatus*) fed a low protein to energy ratios diets supplemented black soldier fly (*H. illucens*) meal.

## 1.3. Objectives

To achieve the aim of the research, the following specific objectives will be addressed:

1. To re-investigate the effect of various P/E ratios on the growth, digestive enzyme activities and body compositions of the spiny lobster juvenile under experimental conditions.
2. To analyse the nutritional contents of black soldier fly meal as a protein source in the diet for *P. ornatus.*
3. To evaluate the optimum inclusion level of defatted black soldier fly meals inclusion on the physiology, hepatopancreas histology and the expression immunology-relatives gene of juvenile lobsters (*P. ornatus*)
4. To evaluate the effect of fish protein hydrolysate supplementation in the experimental diets containing full fat and defatted BSF meal on the growth, survival, histopathology, and body composition of juvenile lobsters.

## 1.4. Significance

The present study should make significant contributions to improving spiny lobster aquaculture by contributing to the understanding of the effective uses of dietary BSF meal and a low P/E ratio diet for spiny lobsters. The specific significances of the present study are outlined as follows.

1. The study will provide new information about the possibility of the use of low P/E ratio diets for spiny lobster aquaculture.
2. Present research will provide nutritional information of black soldier fly meal as an alternative protein source for juvenile lobsters and their suitability in term of the physical properties.
3. The research will provide baseline data about the effect of black soldier fly meal inclusion in the diet for juvenile lobsters and its impact on the physiological and health status of juvenile lobsters.
4. The research will provide information about the effect of fish protein hydrolysate supplementation to the black soldier fly meal based-diet and their impact on the physiological and health status of juvenile lobsters.
5. The study will contribute to sustaining spiny lobster aquaculture by reducing the fishmeal reliance.

# CHAPTER 2: LITERATURE REVIEW

## 2.1. Spiny lobster industry

The global wild-caught lobster, which is mainly from the genus of homarus, panulirus and Nephrops, was stable at roughly 250,000 – 300,000 tonnes from 2012 to 2019 which comprise about 51.3%, 23.0% and 17.3% respectively in 2021 (FAO 2022). The FAO (2023) reported that total landings of spiny lobsters fluctuated between 2012 to 2021 and reached 73,928 tonnes in 2021. Many lobster species were caught, and the largest species that landed is the Caribbean spiny lobster (*Panulirus argus*), reaching 39.65 % of total landings and only 3.66 % or 2.708 tonnes for *Panulirus ornatus* in 2021. Meanwhile, according to the FAO (2023), the production of spiny lobsters from aquaculture reached 3,000 tonnes in 2020 which was mainly produced in Vietnam and Indonesia (FAO, 2023). That production was the highest in the last decades. Both countries adopt a similar method of lobster aquaculture by rearing the puerulus into the market size (Jones 2010).

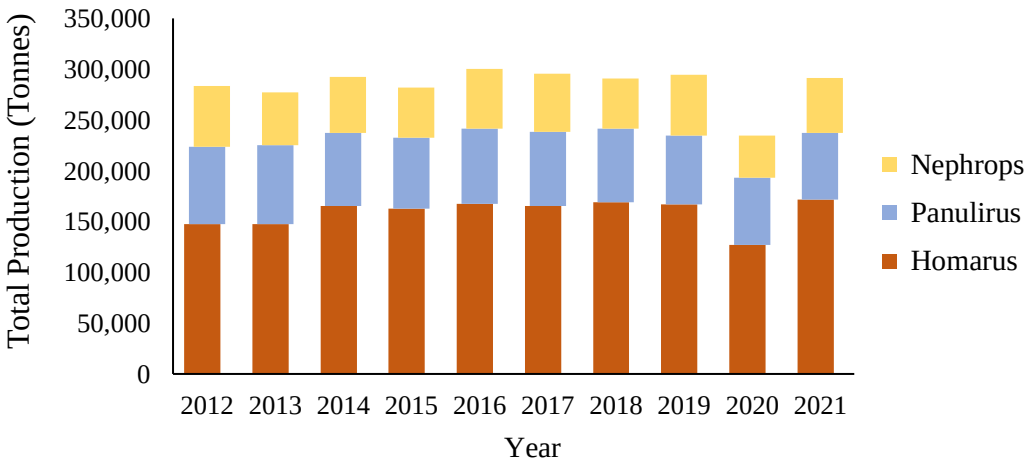



Figure 1. Global wild-caught lobster landings in 2012 – 2021 (FAO 2022)

The common practices of lobster aquaculture consist of the wild-caught swimming puerulus developed in nurseries and growing out in designated floating sea cages (Jones 2009b; Phillips and Matsuda 2011; Priyambodo 2018). Biological

characteristics such as long stage development period during the phyllosoma stages have become problematic technical barriers in the development of spiny lobster hatcheries (Phillips and Matsuda 2011). Therefore, the collection of the wild juveniles at the swimming puerulus stage and small-sized juveniles are more efficient than complete breeding in the hatchery in providing seed for the lobster aquaculture. In Indonesia and several other Asian countries, many studies had been undertaken to investigate the potential for lobsters aquaculture, involving the collected puerulus from natural catches (Kurnia et al. 2017; Ratunil 2017; Syafrizal et al. 2018; Prayitno et al. 2020; Slamet et al. 2020b). During the grow out period, lobster juveniles were fed with fresh finfish, shellfish, shrimp, and small crabs with adjusted portion according to the size of the lobster (Petersen and Phuong 2010; Petersen et al. 2015). Those feeding practices is the most adopted feeding method and very common in lobster aquaculture.

## 2.2. Taxonomy, morphology, and biology of *P. ornatus*

*Panulirus ornatus* (Fabricius, 1798) has several common names including tropical rock lobster, ornate spiny lobster, and ornate rock lobster, depending on their collection location. The classification of *P. ornatus* is as follows:

Kingdom : Animalia
Phylum : Arthropoda
Subphylum : Crustacea
Superclass : Multicrustacea
Class : Malacostraca
Subclass : Eumalacostraca
Superorder : Eucarida
Order : Decapoda
Sub order : Pleocyemata
Infraorder : Achelata
Family : Palinuridae
Genus : Panulirus
Species : *Panulirus ornatus* (Fabricius, 1798)

The ornate tropical rock lobster species can be distinguished from others by it abdominal features. It has smooth and opened abdominal segment is with a colour variation from brown or green. The antennal flagella are ringed. At the anterior close to the base of the pleura, there are large eyespots with an oblique pale streak in the middle (Holthuis 1991). A recent phylogeny analysis of Panulirus genera using mitochondrial genes and cytochrome oxidase subunit confirmed a previous study (Ptacek et al. 2001). This fastest and largest growing species was found to have a body size of 25 to 30 cm and can reach 50 cm. The male and female animals have different biological features, particularly on the body size. The male lobster has a total length of 128 – 452 mm, while a smaller total length was reported on the female lobster with a size from 181 to 190 mm (Rajamani and Manickaraja 1997). Panulirus genera specifically inhabit the shallow waters near the equator line area and have the largest species number. It covered the area from Fiji, New Caledonia, Australia region excluding the Southern area, Papua New Guinea, Japan, Indonesia, India and East Africa (Holthuis 1991).

*Panulirus ornatus* have several stages of development including phyllosoma, puerulus, juvenile and adult lobster. *Panulirus ornatus* have shorter larval duration of only 110 to 150 days and it has 15-20 moult events and 11 body transformations larvae development to reach the puerulus stage (Shanks & Jones, 2014). In culture condition, Sachlikidis (2010) reported the process of egg hatching into puerulus stage of *P. ornatus* as 115 days and involving 24 instars and 11 stages of development (Smith et al. 2009). The phyllosoma stages of this species are also truly short, no more than 5 months. These biological attributes make this species the best candidate for aquaculture purposes (Phillips and Matsuda 2011). The life cycle of the lobster starts from the mating process of broodstock or adult lobster which occurs at a minimum weight of 1,000 g (Vijayakumaran et al. 2009) at the breeding ground. After the mating process, the female lobster will carry fertilized eggs or berried female known as “breeders” or “brooders”. The newly hatch of phyllosoma drift in the ocean current. The puerulus of spiny lobster can be distinguished from the phyllosoma by its body form. Puerulus has a similar form to an adult spiny lobster but is smaller in

terms of size. At this stage, its body has no colour or pigments which makes it transparent. The survival and growth of lobsters is greatly influenced by several factors including feed and environment (Jones 2009a; Arumugama et al. 2020; Jones 2020; Sudewi et al. 2023; Astuti et al. 2024). Natural food in the form of marine bivalves is very popular with lobsters compared to other types of fish. As carnivore crustaceans, lobsters have natural cannibalistic characteristics which greatly affect survival in their life cycle. Apart from appropriate feed, environmental conditions are very important. For *P. ornatus*, the temperature range between 25 to 31°C and salinity of 35 ppt are suitable water quality conditions to achieve optimal growth (Jones 2009a). Even though lobster cultivation has been carried out for several years in various countries, the increase in cultivation production target capacity has not been matched by the availability of suitable feed to date (Nankervis and Jones 2022). Meeting the need for lobster feed from marine bivalves has limitations in terms of production and issues related to sustainability. Therefore, providing feed that suits the needs of lobster cultivation is very important to support the development of lobster cultivation now and in the future.

## 2.3. Nutrition Research in tropical spiny lobster

Understanding the nutritional requirement of the spiny lobster is essential and requires further investigation (Williams 2007, 2009; Francis et al. 2014). In the commercial scale of spiny lobster farming, fresh natural fish is not recommended due to high feed conversion rate (Tuan and Hambrey 2000) and therefore the formulation of aquafeeds for several spiny lobsters was initiated (Glencross et al. 2001; Smith et al. 2003; Ward, Carter, Crear, et al. 2003; Ward, Carter, & Crear 2003).

### 2.3.1. Protein requirement

In aquaculture, protein was the most studied subject due to its vital function in fish. In Table *1*, several study has shown the requirement of protein for spiny lobster species. In the early study of nutritional requirements of the ornate spiny lobster, the optimum crude protein for maximum growth was found to be 474 – 533 g $kg^{-1}$ (Smith et al. 2003). Higher crude protein content was observed to be more optimal for the

same species growing in another experiment the following year. An optimized crude protein requirement study indicated that *P. ornatus* achieved maximum growth and survival when fed 610 g $kg^{-1}$ of crude protein content from fishmeal (Smith et al. 2005). In 2009, the protein requirement for *P. ornatus* was reviewed and it was recommended to formulate a spiny lobster diet with a crude protein content of at least 560 – 580 g $kg^{-1}$ (Williams 2009). The established protein requirement reported by Smith et al. (2003) was then re-examined and resulted in unsatisfactory findings with a specific growth rate of the lobster juvenile being low, at only 0.5% $day^{-1}$. The shortened attractiveness of the tested pellet is responsible for the results. Based on that finding, the protein requirement for *P. ornatus* was then re-evaluated with the addition of bloodworm and krill meal at high crude protein content. Results indicated that the formulation used for the diet is appropriate for optimal growth with specific growth rate coefficients up to 1.38% $day^{-1}$. This growth is comparable with the average growth of spiny lobster juveniles in nature (Irvin and Williams 2009b).

Table 1. Crude protein requirement in spiny lobsters

| Lobster species | Crude protein (%) | References |
|---|---|---|
| *P. ornatus* | 47.53 – 53.3 | Smith et al. (2003) |
| | 61.0 | Smith et al. (2005) |
| | 56.0 – 58.0 | Williams (2009) |
| | 44.0 | Johnston et al. (2008) |
| | 33.4 – 40.5 | Irvin and Shanks (2015) |
| *P. homarus* | 35.5 | Rathinam et al. (2009) |
| | 45.0 | Vijayakumaran (2004) |
| | 46.5 | Prayitno et al. (2020) |
| *P. argus* | 25.0 | Perera et al. (2005) |
| *P. cygnus* | 55.0 | Glencross et al. (2001) |
| *J. edwardsii* | 37.4 | Ward et al. (2003) |

In the younger lobster juvenile, optimal growth can be achieved with an inclusion level of 44% of crude protein in the pelleted diet (Johnston et al. 2008). A lower crude protein inclusion level in the feeds which ranged between 33.40% to

40.5% fed to the juvenile *P. ornatus* resulted in similar growth. However, it was difficult to conclude whether such crude protein inclusion was appropriate since that study was not focused on the nutritional point of view (Irvin and Shanks 2015).

The availability of amino acids in fishmeal has a crucial function in supporting the growth and immune system of the body. However little is known about the amino acid requirements for spiny lobsters. The amino acid requirement for most crustacean species including spiny lobsters is most likely to refer to previous studies (Akiyama et al. 1989; Koshio et al. 1993; NRC 1993). Based on several nutritional studies of lobsters, Nankervis and Jones (2022) developed the minimum essential amino acids requirement for lobsters (Table 2).

Table 2. Essential amino acids requirements for spiny lobster

| Amino acids | Amount required ($g.kg^{-1}$) |
|---|---|
| Arginine | 2.3 |
| Histidine | 0.9 |
| Isoleucine | 1.9 |
| Leucine | 2.8 |
| Lysine | 2.8 |
| Methionine | 1.0 |
| Phenylalanine | 2.2 |
| Threonine | 1.9 |
| Valine | 2.0 |

Research on the amino acid requirement for lobsters may not be important for aquaculture systems that use fresh fish feeds. However, over time, this becomes important when artificial feed is needed for lobster aquaculture. Therefore, research into the need for amino acids in artificial feed for lobsters is an important research base.

### 2.3.2. Lipid

In each stage of lobster development, lipid has been used as an important energy source (Jeff et.al 2001; Jeff et.al 2002; McLeod et.al 2004). Lipid not only

plays an important role as metabolic functions in the body, but also supports the cellular processes. The sparing energy between protein and lipid was developed because of the importance of these ingredients' roles. In crustaceans, roughly 10% of lipid content in the diet is maximum for growth (Cuzon and Guillaume 1997). Some research on lipid requirement in Panuliridae lobsters has shown that the lobsters require 6-10% of lipid (Glencross et al. 2001; Johnston et al. 2003; Smith et al. 2005; Ward 2005; Johnston 2006) (Table 3). In *P. ornatus* juveniles, the levels of lipid and crude protein interact positively with each other. However, the best growth was obtained at a 13% lipid level (Smith et al. 2003).

Table 3. The level of lipid used in nutritional study of lobster species

| Species | Lipid (%) | References |
|---|---|---|
| *P. ornatus* | 13 | Smith et al. (2003) |
| | 11-13 | Smith et al. (2005) |
| | 11-16 | Johnston et al. (2008) |
| | 8-9 | Irvin and Shanks (2015) |
| *P. homarus* | 7.1-7.6 | Rathinam et al. (2009) |
| | 7.07-8.85 | Slamet et al. (2020a) |
| *P. argus* | 6.2-7.0 | Perera et al. (2005) |
| | 9 & 10 | Rodríguez-Viera et al. (2017) |
| | 9 & 10 | Rodríguez-Viera et al. (2014) |
| *P. cygnus* | 6 & 10 | Glencross et al. (2001) |
| *J. edwardsii* | 5 & 9 | Ward, Carter, Crear, et al. (2003) |
| | 12.2-15.5 | Ward (2005) |
| | 11 | Simon and Jeffs (2011) |
| *Sagmariasus verrauxi* | 15 | Shu-Chien et al. (2017) |

## 2.4. Protein to energy ratio in tropical spiny lobster

Nutrition in spiny lobsters becomes a major constraint in this species' aquaculture development (Nelson et al. 2006; Jones 2009b; Francis et al. 2014). A selected fresh natural diet such as blue mussel and frozen seafood with high protein

content was found to be suitable for ornate lobster growth under experimental conditions and became the standard of previous research nutrition foundations (Smith et al. 2005; Irvin and Williams 2006). From acquired baseline references, a high amount of protein and lipid was used in order to meet the quality of that natural diet (Smith et al. 2003; Smith et al. 2005; Williams 2007). Despite gaining the maximum value for growth, the excessive amount of protein and lipid in the feed induces some potential problems including inefficiency of feed utilization, negative environmental impact, and biological effects. A wide variety of protein sources, pellet size, and diet treatments were used to improve the quality of the artificial diet for spiny lobsters (Do Huu and Huong 2014; Kurnia et al. 2017; Marchese et al. 2018). However, results are still unclear for practical implementation.

Some research on nutrition were conducted by evaluating the growth and physiological response on the fish following the treatments of protein to energy (P/E) ratio (Woolley et al. 2010; Li et al. 2012; Taynor 2013; Anizah et al. 2017; Kabir et al. 2019; Khan et al. 2019) and crustacean (Goda 2008; Hu et al. 2008; Venero et al. 2008; Cui et al. 2017; Zhang et al. 2017; Hamidoghli et al. 2018; Méndez-Martínez et al. 2018) as is presented in Table 4. In tropical spiny lobsters, the evaluation of the P/E ratio is poorly understood and has not been investigated thoroughly. Rather than evaluating the P/E ratio, the dietary carbohydrate/lipid ratio has been established in *J. edwardsii* (Johnston et al. 2003). Many nutritional studies on lobsters provide the P/E ratio value of the experimental diets. However, that information was not the main variables of the research tests but were considered as additional information on the tested diets (Johnston 2003; Johnston et al. 2003; Ward, Carter, Crear, et al. 2003; Ward, Carter, & Crear 2003; Smith et al. 2005; Ward 2005). The growth performance of crustaceans was reported to have a positive correlation with the P/E ratio (Smith et al. 2005; Huo et al. 2014). In contrast, Simon and Jeffs (2013) reported that the different P/E ratios in the formulated diet for *Jasus edwardsii* did not affect the mean body weight of lobsters. A formulated diet containing a series of carbohydrate inclusion from maize starch was set to have P/E from 15 to 34.9 g CP $MJ^{-1}$. Although the growth of lobster juveniles was not different after 3 weeks of feeding treatment,

the availability of glucose, glycogen, and soluble protein of high inclusion of maize starch was higher than others. Further, lower energy diets (15.0 g CP $MJ^{-1}$) were more stable in terms of water immersion than higher P/E ratio diets (Simon and Jeffs 2013).

Table 4. The P/E ratio requirement in several decapod crustaceans

| Species | P/E ratio (g $kJ^{-1}$) | CP (%) | References |
|---|---|---|---|
| *P. ornatus* | 29.6 | 61 | Smith et al. (2005) |
| *J. edwardsii* | 15-34.9 | - | Simon and Jeffs (2013) |
| *P. monodon* | 26.65-34.91 | 33-44 | Chuntapa et al. (1999) |
| *L. vannamei* | 21.1 | 34 | Hu et al. (2008) |
| | 17.5 | 35 | Hamidoghli et al. (2018) |
| *L. setiferus* | 28.8 | 40 | Guzman et al. (2001) |
| *M. nipponense* | 16.49 | 33 | Zhang et al. (2017) |
| *M. americanum* | 18.0 | 35 | Méndez-Martínez et al. (2018) |

Over a decade ago, Smith et al. (2005) found that the growth of juvenile *P. ornatus* was optimum when fed a dietary formulated diet containing 47% crude protein compromising P/E ratio of 22.8 g CP $MJ^{-1}$. In that study, the growth of spiny lobster juveniles fed a formulated diet was better than the juveniles fed fresh mussels with an unknown P/E ratio. The growth responses of lobster juveniles tend to improve when the P/E ratio is increased. In recent research on *J. edwardsii*, the inclusion of carbohydrates in the diet was found to be important in maintaining the energy of this species. For comparison, Chuntapa et al. (1999) reported that for the diet of the giant tiger shrimp (*P. monodon*), the P/E ratio of the experimental diets containing 33-44% of crude protein content were between 26.6 to 34.9 g CP $MJ^{-1}$. In addition, the optimum growth of *L. vannamei* and *L. setiferus* was achieved by animals which received a lower P/E ratio than those in the lobster (Guzman et al. 2001; Hu et al. 2008). The juvenile of *L. setiferus* fed with a formulated diet containing 40% crude protein and having 28.8 g CP $MJ^{-1}$ resulted in optimum weight gain. Above that level, the growth of juvenile *L. setiferus* was observed to be depressed. However, carbohydrates as sparing energy resources may not replace protein in the diet

formulation (Guzman et al. 2001). A study on *L. vannamei* revealed that at the crude protein content of 34% and the P/E ratio of 21.1 g CP $MJ^{-1}$, the optimum growth was achieved although the survival rate was similar among the treatments (Hu et al. 2008). In freshwater crustaceans, the optimum P/E ratio for maximum growth was reported to be lower than in brackish and marine crustaceans (Zhang et al. 2017). Under laboratory conditions, the highest growth of *Macrobrachium nipponense* was achieved by including 33% or crude protein in the diet resulting in a P/E ratio of 16.49 g CP $MJ^{-1}$.

### 2.5. Fishmeal replacement in spiny lobsters

Among several nutritional experiments in cultured species, fishmeal replacement has become one of the most researched topics. The optimum fishmeal replacement in fish or crustacean diets in general is highly reliant on the species characteristics. For instance, in carnivore fish species such as salmon, barramundi, and snapper, the fishmeal replacement of up to 50% was observed to have no detrimental effects on the growth. Further, the fishmeal replacement in omnivore fish species is much higher than the lower natural requirement for protein. In decapod crustaceans, fishmeal studies have been studied for over a decade with varying results. For decades, the replacement of fishmeal as a protein resource was intensively studied and considered to be suitable for some species, including spiny lobsters (Williams 2004; Irvin and Williams 2007; Williams 2007; Rathinam et al. 2009; Rodríguez-Viera and Perera 2012; Kurnia et al. 2017; Marchese et al. 2018; Ridwanudin et al. 2018).

A suitable alternative protein ingredient for fishmeal should have well-balanced nutrition contents including micro and macro nutrients and also amino acid content (Khan et al. 2019). In using alternative ingredients for substituting fishmeal in the diet of the fish, several properties aspects were taken into consideration. The physical, nutritional content and biochemical properties of the ingredients are key factors in deciding the use of these materials. Fishmeal was found to be the most suitable protein source ingredient in formulating diets for *P. ornatus* (Irvin and Williams 2007) although the fishmeal quality is also found to be an important

consideration later on (Irvin & Shanks, 2015). Attempts of the fishmeal substitution in the aquafeeds for the aforementioned species using various ingredients is presented in Table *5*.

Table 5. Feeding research in the spiny lobster species

| **Species** | **Protein sources** | **References** |
|---|---|---|
| *P. ornatus* | Mussel | Smith et al. (2005) |
| | Krill meal | Johnston et al. (2008), Smith et al. (2005) |
| | Squid | Marchese et.al (2018), Johnston et al. (2008) |
| | Fish head meal | Kurnia et al. (2017) |
| | Crustacean meal | Irvin and Williams (2007), Irvin and Williams (2009a) |
| | Defatted fishmeal | Irvin and Williams (2009b) |
| | Artemia | Johnston et al. (2008) |
| | Trash fish | Irvin and Shanks (2015) |
| *P. ornatus & J. edwardsii* | Soybean meal, lupin meal, wheat | Irvin & Williams (2007), Ward et.al (2003b) |
| *P. argus* | Gastropods | Díaz-Iglesias et al (1991) |
| | Artemia | Lellis (1992) |
| | Squid | Perera et al. (2005), Rodríguez-Viera and Perera (2012) |
| | Clam meal | Perera et al. (2005) |
| *P. homarus* | Frozen seafood, fresh seafood | Manambrakat and Kumar (2010), Cox and Davis (2009), (Astuti et al. 2024) |
| | Clam & squid meal | Rathinam et al. (2009) |
| | Mussel | Giri et al. (2023) |

Various alternative protein sources both from marine and terrestrial plants have been studied for potential usage in spiny lobster diets (Ward, Carter, & Crear 2003; Irvin and Williams 2007; Johnston et al. 2008). Mussel meal and prawn are two favourable selected ingredients for fishmeal substitutes due to their high crude protein digestibility value. On the other hand, plant protein sources such as lupin flour and wheat gluten were also potentials (Ward, Carter, & Crear 2003). Years later, Irvin and Williams (2007) reported that the crude protein content in squid meals and krill meals was found to be higher than in other ingredients. In addition, the crude protein

apparent digestibility of krill meal was also comparable to mussel meal. However, it is not suggested to use squid meal since its protein digestibility is low.

Johnston et al. (2008) reported a series of nutritional studies on the first larval stages of *P. ornatus*. That nutritional study incorporated the use of squid meal, artemia, green mussel, and a combination of artemia and green mussel. In one of their experiments, the fishmeal replacement using squid is suboptimal for phyllossomata growth resulting in only 2.13 cm of total length and 1.14 cm carapace length which was significantly lower than phyllosomata fed with a fishmeal-based diet. An updated nutritional study for spiny lobster was conducted intensively in *P.ornatus* and incorporated various feeding treatments (Irvin and Shanks 2015). The raw material quality of fishmeal influences the growth of lobsters. The inclusion of high-quality fishmeal was found to result in better growth in the lobster juveniles compared to a formulated diet using unknown-quality fishmeal. At a similar crude protein and lipid level of 41% and 8% respectively, spiny lobsters fed with high-quality fishmeal attained significant weight gain at 126% after 42 days of trial. In addition, the inclusion of fresh mussels as fishmeal substitutes in the diet range between 149 g $kg^{-1}$ to 198 g $kg^{-1}$ improved lobster weight gain. Mixed various ingredients were also found to be more effective than solely trash fish diets to promote the growth of lobster juveniles. Further, it is recommended that the combination of fresh mussel and fish are important for the growth of lobsters (Irvin and Shanks 2015).

The study of fishmeal replacement in spiny lobsters was not further investigated until a recent study involving the addition of krill meal as a diet supplement to the spiny lobster diet was conducted (Marchese et al. 2018). It has shown that krill meal may contribute to the development of early juvenile *P. ornatus* reared in a communal situation. The inclusion level of up to 10% of the krill meal in the diet of early stages of *P. ornatus* improves growth rates although growth rates are still below the growth rates of spiny lobster fed with blue mussel. The results of that study supported previous studies in terms of the incorporation of krill meal in the tested diets (Smith et al. 2005; Irvin and Williams 2007). The inclusion of 300 g $kg^{-1}$ of krill meal containing (610 g $kg^{-1}$) resulted in an average weight gain of up to 0.91

g week$^{-1}$. In contrast, the average weight gain of the spiny lobster fed with natural blue mussels was only 0.42 g week$^{-1}$ (Smith et al. 2005).

## 2.6. The roles of Black Soldier Fly (BSF) meal in aquafeed to the health and immunity of crustaceans

Apart from being used as protein sources in the diet of freshwater species, several recent studies involving BSF in the formulated diets for marine species have been reported (Belghit et al. 2019; Wang et al. 2019; Abdel-Tawwab et al. 2020; Fisher et al. 2020; Li et al. 2020). The growth and immunological status of the Atlantic salmon (*Salmo salar*) was improved following complete fishmeal replacement using BSF meal. The amino and fatty acids were still found in the diet even though the fishmeal as the protein source was replaced totally using BSF meal (Belghit et al. 2019). Although a number of research on the incorporation of BSF meal in aquafeed have been reported, similar nutritional study is scant in crustaceans (Table 6). The first reported study of fishmeal replacement in marine crustaceans using BSF was conducted on white Pacific shrimp (Cummins et al. 2017). In that study, six pelleted diets having increment BSF levels of 0, 7, 14, 21, 26 and 36% and total crude protein ranging between 401.5 g kg$^{-1}$ to 426.6$^{-1}$ were evaluated. After 63 days of culture under laboratory conditions, the growth of shrimp juveniles was analysed. The increase in BSF meals was found to be ineffective in improving the growth. Rather than improving the growth rate, the excessive BSF meal substitution decreased the weight gain of the animals and increased the feed conversion coefficient. That results were believed as the results of the low digestibility and palatibility of the BSF in the diet. The juvenile shrimp achieved their optimum growth with the maximum inclusion level of BSF meal at 7% of substitution (Cummins et al. 2017). In another nutritional study, the BSF meal was suitable as the complement for non-fishmeal protein sources such as poultry by-product meal which resulted in optimum growth of *C. cainii* (Foysal et al. 2019). Freshwater lobster juvenile fed an experimental feed containing 110 g kg$^{-1}$ of BSF meal has higher weight gain than those fed experimental feed without BSF meal. Besides, the combination of both

alternative ingredients has also improved the immunological genes of freshwater lobster juveniles (Foysal et al. 2019).

Together with BSF, yellow mealworm (*Tenebrio molitor*) has also been used as a fishmeal replacement in fish diets (Janssen et al. 2017; Henry, Gai, et al. 2018; Henry, Gasco, et al. 2018; Iaconisi et al. 2018; Thévenot et al. 2018; Ido et al. 2019). Dietary protein sources from mealworm (MW) in the diet of the red seabream (*Pagrus major*) are found to be suitable. An optimum specific growth rate was achieved after 4 weeks of feeding treatment using 65% of dietary MW. Furthermore, this replacement has also improved the immunity of the fish when challenged with the pathogenic bacteria *Edwardsiella tarda* (Ido et al. 2019). It has been reported that yellow catfish (*Pelteobagrus fulvidraco*) can tolerate the high level of BSF meal in the diet. Up to 75% of fishmeal can be substituted using MW without negative effects on growth and general immunology conditions. In addition, the bacterial resistance of catfish fed with dietary MW was improved (Su et al. 2017). Although the presence of fishmeal in the fish diet can be replaced completely by MW, the maximum amount of inclusion should not exceed 25% to maintain the quality of the fish flesh (Iaconisi et al. 2018).

Table 6. Fishmeal replacement using insect meal in some crustaceans.

| Insects | Inclusion (%) | Results | References |
|---|---|---|---|
| *T. molitor* | 50% | Improve immunocompetence | Choi et al. (2018) |
| *T. molitor* | 100% | There was no adverse effect | Panini et al. (2017) |
| *T. molitor* | 50% | Optimum growth and FCR | Motte et al. (2019) |
| *H. illucens* | 29% | Similar to control | Cummins et al. (2017) |
| *H. illucens* | 12% | Enhanced immune system | Foysal et al. (2019) |

Yellow mealworm (*T. molitor*) has been effectively added as a dietary supplement to the diet of Pacific white shrimp, *L. vannamei* (Choi et al. 2018; Motte et al. 2019). A proximate composition analysis which has been performed by Motte et al. (2019) reveals that mealworm has comparable nutritional content to fishmeal, including crude protein (74.8%), crude fat (12.6%), crude fibre (4.7%), and moisture (3.9%). Those nutritious feed ingredient sources offer the potential for complete fishmeal substitution for *L. vannamei*. In an 8-week feeding experiment, shrimp fed with 50% mealworm meal substitution achieved the highest specific growth rate (2.59 % $g.day^{-1}$). Together with two other treatments (25% and 100% fishmeal replacement), this value is significantly higher than 0% for fishmeal replacement (Motte et al. 2019). This finding is in accordance with a previously reported feeding trial on the same species (Choi et al. 2018). A series of diets containing fishmeal of 0, 25, 50 and 100% replacement using mealworm was conducted on a semi-intensive culture scale. The feeding treatments at 50% fishmeal replacement using mealworm meal resulted in the optimum growth rate of 2.79 % $day^{-1}$ (Choi et al. 2018). Although many published papers reported the potential use of these ingredients in replacing fishmeal in some cultured fish and crustaceans (Sánchez-Muros et al. 2016), the use of this ingredient in tropical spiny lobster is unknown.

## 2.7. Black soldier fly meal as alternative protein sources for crustaceans

After Ward, Carter and Crear (2003) and Irvin and Williams (2007), little attention was directed on the search for alternative ingredients as protein resources for the diet of the tropical spiny lobster. Meanwhile, several studies investigated the potential use of insect ingredients for aquafeed (Riddick 2014; Henry et al. 2015; Sánchez-Muros et al. 2016). Several insects species have been used in the aquaculture industry including housefly, black soldier fly, mealworm, cricket or grasshopper and silkworm (Makkar et al. 2014). Among the cultivated insect species, black soldier fly *(Hermetia illucens)* share 13% of the total insects used in aquaculture (Sánchez-Muros et al. 2016). The proximate composition of BSF in several feeding experiments in fish is presented in Table 7. The nutritive value of BSF was determined by the stage, processing method (Zozo et al. 2022; Zulkifli et al. 2022), and substrates

(Jucker et al. 2017; Julita et al. 2019; Shumo et al. 2019; Bortolini et al. 2020). The proximate composition of BSF varies and there was not any standard for it. In general, the full fat BSF has higher lipid or fat content than defatted BSF and compromise the crude protein in it.

Table 7. The proximate composition of the black soldier fly (g/100g dry matter)

| Proximate composition | References (*) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) ¶ | (5) | (6) ¶ | (7) | (8) | (9) ¶ | (10) ¶ | (10) † |
| Protein | 48.2 | 32.1 | 42 | 56 | 41.6 | 52 | 42.6 | 35 | 55.9 | 45.8 | 56.1 |
| Lipid | 25.7 | 32.2 | 32 | 11.8 | 23.2 | 15.1 | 23 | 29.8 | 8.5* | 25.8 | 4.8 |
| Fibre | 9.9 | NA | NA | NA | 7.6 | NA | 10.8 | 7.9 | NA | NA | NA |
| Moisture | 7.1 | 8.5 | NA | NA | NA | 10 | 8.2 | NA | NA | 4.4 | 6.5 |
| Ash | 8.3 | 13 | 9.25 | NA | 11.6 | 7.3 | 10.5 | 5.3 | 7.6 | NA | NA |
| NFE | 7.9 | NA | NA | NA | 10.8 | NA | 4.9 | 22.1 | NA | NA | NA |

*) (1) Zulkifli et al. (2022), (2) Hu et al. (2020), (3) Weththasinghe et al. (2021), (4) Fisher et al. (2020) , (5) Devic et al. (2018), (6) Cummins et al. (2017) , (7) Adeoye et al. (2020), (8) Rawski et al. (2020), (9)Biasato et al. (2019), (10) Zozo et al. (2022). ¶= fullfat, †=defatted, NA = not available

In full fat BSF, the crude protein content only ranged between 32.1 to 48.2 % of the total dry matter, while its content in defatted BSF ranged between 52.0 to 56.1% (Cummins et al. 2017; Biasato et al. 2019; Fisher et al. 2020; Zozo et al. 2022). Excessive lipid is found in the full fat BSF with a minimum value of as much as 20%. The lipid content of the full fat BSF was up to 32.2 % (Hu et al. 2020) whereas only less than 10% of lipid content was found in the defatted BSF (Biasato et al. 2019; Zozo et al. 2022). Although the high lipid content of BSF may have drawbacks for cultured fish, it can be used as a lipid source in diet formulation (Xu et al. 2020).

The evaluation of amino acid requirement is a particular necessity for fish feed formulation to obtain the most suitable composition (Xing et al. 2023). The fatty acids content in the BSF has been reviewed as feed sources (Abd El-Hack et al. 2020; Lu et al. 2022) or as alternative energy sources (Franco et al. 2021). Therefore, the quantity of the amino acids of the feed ingredients is a necessity. As the BSF has mixed value proximate composition, its amino acids also varies in several studies (Table 8 & Table 9).

Table 8. Indispensable amino acids of the black soldier fly (*H. illucens*) (g/100g)

| Amino acids | References | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) |
| Arginine | 1.3 | 1 | 2.5 | 2 | 2.3 | 2 | 3 | 2.5 | 2.2 | 2.4 | 2.6 | 5 | 5.4 | 1.6 |
| Histidine | 1.7 | 1.5 | 2.8 | 1.4 | 1.5 | 1.2 | 1.9 | 3.1 | 0.9 | 0.9 | 1.6 | 3 | 3.2 | 0.8 |
| Isoleucine | 2.4 | 0.6 | 2.4 | 1.7 | 1.9 | 2.7 | 2.2 | 3 | 1.8 | 2.2 | 2.2 | 4.9 | 4.7 | 1.5 |
| Leucine | 3.4 | 1.2 | 3.6 | 2.9 | 3.2 | 2.9 | 4.2 | 1.4 | 3.3 | 3.5 | 3.6 | 7.2 | 7.8 | 2.9 |
| Lysine | 3.9 | 0.8 | 3.6 | 2.3 | 2.7 | 2.7 | 3.8 | 2.6 | 2.5 | 3.6 | 3.7 | 4.8 | 6.8 | 2.1 |
| Methionine | 1.1 | 1.5 | 1.1 | 0.7 | 0.6 | 0.7 | 1.2 | 0.7 | 0.2 | 2.1 | 1.2 | 1.9 | 2.1 | 0.6 |
| Phenylalanine | 2.1 | 1.2 | 2.1 | 1.7 | 1.6 | 1.7 | 2.3 | 0.7 | 1.7 | 1.7 | 2.3 | 3.9 | 7.7 | 1.5 |
| Threonine | 1.8 | 1.1 | 1.9 | 1.6 | 1.7 | 1.7 | 2.4 | 2 | 1.6 | 2.5 | 2.2 | 4.6 | 4.4 | 1.3 |
| Tryptophan | 0.9 | 1.3 | 3.1 | 0.6 | 0.5 | NA | 0.9 | 3.5 | NA | NA | NA | NA | NA | 0.7 |
| Valine | 2.9 | 0.5 | 3.1 | 2.4 | 2.6 | 2.6 | 3.7 | 2.2 | 1.8 | 3.9 | 3.7 | 5.9 | 6.7 | 1.9 |

(1)Adeoye et al. (2020), (2) Yildirim-Aksoy et al. (2020), (3) Zulkifli et al. (2022), (4) Spranghers et al. (2017), (5) Cummins et al. (2017), (6) Devic et al. (2018), (7) Fisher et al. (2020), (8) Biasato et al. (2019), (8) Hu et al. (2020), (9) Lan et al. (2022), (10) Li et al. (2017), (11) Magalhães et al. (2017), (12) Rawski et al. (2020), (13) Weththasinghe et al. (2021). NA = not available

Table 9. Dispensable amino acids of the black soldier fly (*H. illucens*) (g/100g)

| Amino acids | References | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
| Alanine | 1.3 | 3.1 | 2.5 | 3.7 | 2.5 | 2.4 | 3.3 | 7.7 | 2.4 |
| Aspartate | 1.7 | 5.1 | 3.7 | NA | 4.1 | 4.5 | 5.2 | 9.3 | 3.2 |
| Cysteine | 2.4 | 0.2 | 0.3 | 0.8 | 0.1 | 0.5 | NA | 2.4 | 0.2 |
| Glutamine | 3.4 | 6.1 | 4.2 | 6.4 | 6.1 | 6.8 | 5.5 | 11.6 | 4.2 |
| Glycine | 3.9 | 0.2 | 2.3 | 3.5 | 2.1 | 2.4 | 2.6 | 5.1 | 1.5 |
| Proline | 1.1 | 2.9 | 2.3 | 3.4 | 1.9 | 3.1 | NA | 5.9 | 1.9 |
| Serine | 2.1 | 2.1 | 1.7 | 2.4 | 1.7 | 2.2 | 2 | 7.1 | 1.2 |
| Tyrosine | 1.8 | 3.1 | NA | 3.8 | 0.8 | NA | 3.4 | 5.8 | 1.8 |

(1) Adeoye et al. (2020), (2) Zulkifli et al. (2022), (3) Spranghers et al. (2017), (4) Fisher et al. (2020), (5) Hu et al. (2020), (6) Lan et al. (2022), (7) Li et al. (2017), (8) Magalhães et al. (2017), (9) Weththasinghe et al. (2021). NA = not available.

### 2.8. The role of protein hydrolysate in the health status of crustaceans

Along with the increasing levels of fish consumption, more and more fish waste is generated from processing activities and direct consumption of fish. In several countries, the waste from fish processing activities is further processed to become fishmeal. That fishmeal is then used as a protein source ingredient in the manufacturing of aquafeed livestock feed. The remaining materials from processing come from scales, bones, heads, and internal organs of fish with various nutritional value. In addition, the nutritional quality of the remaining fish processing waste can be improved through an enzymatic process, producing protein hydrolysates or fish protein hydrolysate (FPH) in dry or liquid forms. However, some researchers also use fresh fish as a raw material for FPH. The hydrolysis process can be carried out using enzymatic methods or chemical methods. The two processes are distinguished based on the differences in the hydrolysis process. The hydrolysis process involves the decomposition of free peptides derived from raw materials. With this process, peptides are expected to have a smaller size so that they are expected to be more

easily digested in the digestive system of animals. The hydrolysis process requires temperature, pH, and certain enzymes such as alcalase, protease, trypsin, and pepsin to breakdown the short-chain peptide in the hydrolysis material. Thus, the advantages of FPH make it included in the feed formulation in aquaculture, increasing the feed intake and feed utilization which promotes the growth performance of certain fish species.

The fish protein hydrolysate can be produced from a variety of ingredients such as shrimp by-product (Arredondo-Figueroa et al. 2013), krill (Córdova-Murueta et al. 2002), fish waste (De et al. 2020), tuna (Hernández et al. 2011; Nguyen et al. 2012) and mollusc (Zhou et al. 2016) with mixed results in terms of quality or its effectiveness in providing nutrition. In general, FPH has been used as the improvement agent of feed ingredient in feed formulations. The fishmeal replacement in several fish or crustacean species resulted in sub-optimal growth due to the low palatability of the feed. To solve those problems, the incorporation of FPH in the diet formulation was believed to improve the feed quality. While the application of FPH in aquadiets for finfish have been widely reported, its use in crustaceans is progressing with mixed results (Córdova-Murueta et al. 2002; Hernández et al. 2011; Nguyen et al. 2012; Arredondo-Figueroa et al. 2013; Niu et al. 2014; Zhou et al. 2016; De et al. 2020). The utilization of FPH has been reported in freshwater (Arredondo-Figueroa et al. 2013) and saltwater crustacean species (Nguyen et al. 2012; Zhou et al. 2016; De et al. 2020). The inclusion of shrimp-head protein hydrolysate improves the growth and maintains the survival rate at the optimum level of new hatchling freshwater crayfish, *C. quadricarinatus* (Arredondo-Figueroa et al. 2013).

The growth of crayfish hatchlings fed 5% FPH supplementation achieved a significant growth rate and adverse effects were observed when inclusion beyond that level is applied. That result is in line with the individual feed consumption rate of the crayfish. This indicates that there is a direct impact on the feed intake that finally increases the growth of young crayfish. A similar trend was also obtained by a study conducted by Niu et al. (2014). Rather than improving growth performance, the excessive FPH in shrimp feed led to a decrease in the growth performance of *L.*

*vannamei* post larvae and it is recommended that the inclusion of FPH should be at 21.22%-26.35%.

Table 10. The application of fish protein hydrolysate in crustaceans

| Type of PH | Effects | References |
|---|---|---|
| Fish and shrimp | No significant growth performance was observed in *P. homarus* with 3% supplementation | Astuti et al. (2023) |
| Shrimp head | Improved growth efficiency of *C. quadricarinatus* at 5 % inclusion | Arredondo-Figueroa et al. (2013) |
| Krill | Low supplementation (3%) in the feed of *P. vannamei* resulted in the growth enhancement. | Córdova-Murueta et al. (2002) |
| Fish waste | Improvement the production *of P. vannamei* with 20 ppm supplementation | De et al. (2020) |
| Tuna | Growth performance of *L. vannamei* increases | Hernández et al. (2011) |
| Tuna | Improvement on the zootechnical parameters of *P. vannamei* | Nguyen et al. (2012) |
| Squid, scallop | No significant growth performance observed in *L. vannamei* (3%, 6%, 9%) | Zhou et al. (2016) |

In addition, low inclusion levels of FPH also promoted the digestive enzyme activity of trypsin (Córdova-Murueta et al. 2002; Niu et al. 2014). In addition, at the field scale, the application of FPH increases pond productivity by improving the abundance of plankton which promotes the growth, survival rate and total biomass of *L. vannamei* (De et al. 2020). Nguyen et al. (2012) reported that FPH from tuna not only improves the growth of shrimps but also the zootechnics of the shrimps. Apart from the positive results reported by the mentioned studies regarding the utilization or use of FPH in fish or shrimp feed, several other studies also reported that the administration of FPH supplements did not have any effect on the physiology or immunology of fish (Zhou et al. 2016).

# CHAPTER 3: METHODOLOGY

## 3.1. General

This chapter briefly describeds the general materials and methods used in the experiments. The nutritional study including diet preparation and experimental set was conducted at Jakarta Fisheries University Mariculture Station (JFUMS) in Banten, Indonesia. The analysis of proximate composition, mineral composition, feed composition, amino acids, fatty acids, and histopathology were analysed at IPB University Nutrition Laboratory, SIG Laboratory and Fish and Environment Laboratory Indonesia. The gene expression analysis was performed in the Fish Quarantine and Inspection Laboratory, Indonesia. This chapter also presents general procedure for diet formulation, sample collection, data collection, biochemical analysis, digestive enzyme activities analysis, immune-related gene eexpression analysis and statistical analysis.

## 3.2. Experimental animal and Facility

In each feeding experiment, puerulii of tropical spiny lobster were bought from local fishers, land-transported and acclimated for 1-2 weeks or until transformed into pigmented juveniles. During the acclimation, the lobster juvenile was unfed. The healthy juvenile lobsters were selected and graded based on their weight to be used as experimental animals. The juvenile lobster were also measured in term of total length as the initial length data. In each experiment, fibreglass aquaria with the dimensions of 30 cm × 30 cm × 60 cm were used (Figure *2*). Each of the aquaria were equipped with a recirculation water system to maintain the water quality. In each feeding experiment, acclimated juvenile lobsters were distributed to designated plastic baskets as their cage during the experiment to keep them separated from each other. Continuous aeration and mechanical water filtration systems were provided to maintain the water quality. In each feeding experiment, the lobster juveniles were fed four times per day until satiation. The duration of the feeding experiment were a minimum of 8 weeks.

Figure 2. Feeding experimental set-up (aquaria dimension 30 cm x 30 cm x 60 cm)

### 3.3. Formulated feed preparation

The tested feed was produced according to different formulation for each feeding experiment using formulated diet software, LIVE software version 1.52 from Live Informatics Company limited (Thailand). All diet ingredients were collected from the local supplier, including fishmeal, commercial full fat BSF, defatted BSF, and liquid fish protein hydrolysate. In the first experiment, six different feeds having P/E ratio between 21 to 26 mg $kJ^{-1}$ were formulated using fishmeal as the main protein sources. Based on the findings of the nutritional analysis of BSF as alternative protein sources, feed formulation was performed in the third study using these feed ingredients where defatted BSF was used as the main protein source. In the third study, fish meal was replaced with defatted BSF at 25%, 35% and 50% substitution. In the fourth feeding experiment, the feed formulation was based on the suitable level deffated BSF substitution in the third experiment. The feed was formulated with the combination of defatted BSF, fullfat BSF and protein hydrolysate as the protein sources, replacing fish meal. All diet ingredients were grounded, mixed with an appropriate amount for each feeding experiment. The experimental diet were formulated using a mechanical semi-automatic extruder (Oxone OX-861N, Indonesia) and the final product were stored at -20°C until used.

### 3.4. Sample collection

All tested diets from each feeding experiment were analysed for their proximate compositions including crude protein, crude fat, ash, moisture and fibre. Additional amino acids analysis was performed in the first and the third feeding trial.

While fatty acid analysis was performed in the fourth experiment. At the beginning, in the middle, and at the termination, three randomly selected lobster juveniles were measured in terms of survival, wet weight, carapace length, total length, and moulting rate. After the completion of the feeding experiment, all those parameters were re-analysed. An additional quantitative polymerase chain reaction (qPCR) analysis was performed to investigate the gene expressions of the lobster juvenile in the third feeding experiment. In the thrid and fourth feeding experiment, histological analysis was performed.

## 3.5. Data collection

### 3.5.1. Evaluation of growth performances

In general, at the end of the feeding trial, several physiological responses of lobster were assessed, including:

(1) Survival rate (%) = 100 × (final number of lobster/initial number of lobster)

(2) Weight gain (%) = 100 × (final wet weight (g)–initial wet weight (g))

(3) Specific growth rate (% gr $day^{-1}$) = 100 × (ln final wet weight (g)–ln initial wet weight (g))/days

(4) Moulting rate = 100 × (number of moulting lobster/total number of lobster)

(5) Moult interval (days) = duration in days between two consecutive moults

(6) Length increment = 100 × (final body length - initial body length)/final body length

(7) $M_{50}$= the days taken for 50% of the lobster population for moulting.

### 3.5.2. Biochemical analysis

In this study, not all feeding experiment have the similar biochemical analysis. The general chemical analysis was performed in this study including the proximate composition of crude protein, lipid/fat, ash, moisture, carbohydrate, enzyme activity, amino acids, and fatty acids following the procedure:

a. Crude protein

The crude protein content in the diet, feed ingredients and whole body of juvenile lobster were analyzed using the Kjeldahl method. The crude protein was calculated by multiplying the percentage of nitrogen with a coefficient of 6.25 (AOAC 2005b).

b. Total Lipid

The lipid content was extracted through the mixing of chloroform and methanol at a ratio of 2: 1. The solution was then filtrated and evaporated using a vacuum as described in an established protocol (AOAC 2005a).

c. Ash

The ash content was determined by an established protocol Ismail (2017) by heating 1-2 g of samples using a furnace at 550 ºC for 24 hours. The percentage of ash was calculated by weighing the remaining amount of the samples after the heat process.

d. Moisture

The moisture content was determined by heating the samples at 110 ºC ± 1 ºC for 2 hours. The percentage of moisture content was calculated by deducting the final weight from the initial weight of the sample (Nielsen 2017).

e. Carbohydrate/fibre

The crude fiber was measured according to established protocol (BeMiller 2017) using gravimetric method as the residue of the sample using $H_2SO_4$ 1.25% and NaOH 1.25% solution. The insoluble residue is collected through filtration, then it is dried, weighed, and incinerated to account for any mineral contamination in the fiber residue.

f. Amino Acids

The amino acid content was measured using a High-Performance Liquid Chromatography method according to Babu et al. (2002). 10 mL of HCL (6N) was added to the samples before the heating process at 100°C for 24 hours. After the heating process, samples were filtered and 25 µL of the product was transferred to the tubes. A 25 µL dehydration solution was added to the tube to

dehydrate the samples using a vacuum. When the vacuum process was completed, a 25 µL of derivatives solution was added to the tube and left at room temperature. After 20 minutes at room temperature, 25 µL of sodium acetate buffer was added then the samples were injected into the HPLC machine.

g. Fatty acids

The fatty acids content analysis was performed using the gas chromatography method as described by Ratnayake et al. (2006). The peak of the chromatograph was determined by comparing the retention time of every fatty acid component from samples and references.

h. Mineral content

The analysis of mineral content, encompassing calcium, sodium, magnesium, zinc, iron, manganese, and copper, was conducted utilizing the Atomic Absorption Spectroscopy method, following a standardized protocol as outlined by Paul et al. (2014).

i. Percent differences

The percentage variances in the proximate composition, amino acids, fatty acids, and minerals were assessed by applying a well-established calculation method to compare differences between defatted and full-fat BSF (Cummins et al. 2017) as follows :

$$\% \text{ differences} = (TP_{DF} - TP_{FF})/ TP_{FF} \times 100$$

j. Water quality measurement

Water quality including dissolved oxygen, pH, temperature, and TDS were monitored using digital water quality instrument Horiba (U-5000, Japan).

### 3.5.3. Digestive enzyme activity analysis

a. Amylase

In this study, the amylase enzyme activity of the lobster was assessed according to Bergmeyer’s method (Mulyasari et al. 2018). The amylase enzyme was taken from the lobster's whole-body. The analysis begins with the

sample preparation by mixing of the samples with soluble starch in citrate buffer (pH 5.7), followed by the incubation (32ºC, 30 minutes) process before heating at 100 ºC for 10 minutes. To determine the amylase enzyme activity, the processed samples were analysed using spectrophotometer at the absorbance level of 578 nm.

b. Protease

The protease activity was determined using spectrophotometer method where each lobsters samples were mixed with boric acid buffer (0.01 M, pH 8.0), casein substrate (20 mg $mL^{-1}$ pH 8.0), HCl (0.05 mg $mL^{-1}$) and standard tyrosine (5 mmol $L^{-1}$), The mixture underwent an incubation process in a water bath at 37ºC for 10 minutes. Following incubation, 0.1 M TCA was introduced to the sample tubes and left to incubate at 37ºC for an additional 10 minutes, followed by centrifugation at 3,500 rpm for 10 minutes. The resulting mixture was stored at 37ºC for 20 minutes before measuring absorbance at 578 nm.

c. Lipase

Meanwhile, the lipase activity was measured according to an established protocol (Tietz and Fiereck 1966).

d. Superoxidase dismutase Activity

The Superoxidase Dismutase (SOD) activity was determined using the SOD test kit (Abcan, UK). The process was initiated by preparing lobster samples approximately 0.3 g and diluted into 10 mL sulphuric acid 10M and centrifuged. Before the incubation process, a 20 µL working solution containing 20 µL SOD Assay Buffer and 50 µL SOD Enzyme solution in 100 mL cool PBS solution was prepared and distributed to the sterile tubes. The supernatant from the dilution process was then added to the prepared tube before 20 minutes of incubation. To finish the reaction, 50 µL of Butylated hydroxytoluene was added to each tube, followed by measurement using a spectrophotometer at an absorbance of 550 µm.

### 3.5.4. Histopathology analysis

At the end of the feeding trial, the intestine and hepatopancreas of juvenile lobster were collected for histopathological analysis using hematoxylin-eosin staining according to an established protocol from Bell and Lightner (1988). At the beginning of the sample analysis, all samples were preserved using Davidson's solution for 24 hours and then transferred to 70% ethanol solution. A small cut of the abdomen and cephalothorax from each sample were placed in cassettes for further process dehydration, clearing and infiltration in an automatic tissue processor (Tissue-Tek VIP 5 Jr. 5903, Japan). The sequences of these processes include the series of ethanol solutions (70%, 80% and 95%), absolute ethanol solution, xylene, and paraffin. The process using each solution was taken twice and took two hours. The blocking process of the samples was using the embedding console system machine (Tissue-Tek VIP 5 Jr. 5903, Japan). Each of the samples was then trimmed and sliced using a microtome (Leica RM2125, US) with a thickness of 4-5 µm. After the completion of the slicing, the thin layers of samples were then stretched in a flotation bath at 45ºC and were then placed on glass slides and dried. Before the staining process, deparaffinization of samples was carried out using a hotplate at 62ºC. The staining process was performed using xylene, absolute ethanol, ethanol solutions, hematoxylin, bluing reagent, and eosin with certain procedures. After the completion of the staining process, the samples on the glass slides were then covered with a cover glass. Overall, a total of six pathology sections were observed at 40× magnification using a light microscope (Carl Zeis Primo Star, US). The height of the villus and muscle thickness of the intestinal tissues was measured using a Digital ruler (Adobe Photoshop, US).

### 3.5.5. Immune-relative gene expression analysis

In this study, not all feeding experiments were applied for immune-relative gene expression analysis. Only juvenile lobsters harvested from Chapter 7 were taken for the gene expression analysis. Two juvenile lobsters from each treatment were chopped into small pieces and stored in a 1.5 mL PCR tube, followed by the addition of 500 µL of RNA extraction solution and grinding process. Total RNA was extracted by using the RNA extraction solution kit (IQ-2000TM Genereach, Taiwan). After 5

minutes at room temperature, 100 µL of chloroform was added. The sample was then vortexed for 20 s and centrifuged at 12.000 rpm for 15 minutes. At the end of the centrifugation, 200 µL of a clear layer of upper solution was removed to a new tube and immediately added with 200 µL isopropanol. After a short vortexing, the sample was re-centrifuged at 12.000 rpm for 10 minutes and the isopropanol discharged at the end of the process. The RNA pellet was then washed using 500 µL ethanol (75%), spun down and centrifuged at 9,000 rpm for 5 minutes. The RNA pellet was diluted using DEPC ddH2O 50 µL and stored at -20°C for cDNA analysis using the SensiFAST cDNA Synthesis Kit (Bioline, Australia). The total reaction volume was 20 µL consisting of 4 µL 5x TransAmp Buffer, µ1 L reverse transcriptase, 15 µL free-water RNase and 3.0 g total RNA. All materials were then mixed slowly and then the solution in the microtube was processed using a thermal cycler at 25°C for 10 minutes, 42°C (15 minutes), and 85°C for five minutes for inactivation. The cDNA solution was then placed on ice to stop the synthesis reaction and stored at a minimum -20°C for further analysis.

Table 11. Primers used for juvenile lobster immunology following fishmeal replacement with defatted BSF meal.

| Primer | Primers sequence Forward (F) | Primers sequence Reverse (R) |
|---|---|---|
| IL-1β [1] | GTTTACCTGAACATGTCGGC | AGGGTGCTGATGTTCAGCCC |
| IL-10 [1] | CAGTGCAGAAGAGTCGACTGCAAG | CGCTTGAGATCCTGAAATATA |
| IL-17F [1] | GTCTCTGTCACCGTGGAC | TGGGCCTCACACAGGTACA |
| β-actin [1] | TTGAGCAGGAGATGGGAACCG | AGAGCCTCAGGGCAACGGAAA |
| proPO[2] | GAGATCGCAAGGGAGAACTG | CGTCAGTGAAGTCGAGACCA |
| GPO[2] | TTTTTCCGTGCAAAAAGGAC | TAATACGCGATGCCCCTAAC |
| ALFHa 1[2] | CAGTCGTTCTGGTGTTGTTGGGAA | TTGTTGGGCATCCCTCTCGGTAT |
| ALFHa 2[2] | AGACTACCACTGACTTCGTGAGGA | TCTCGGGATGATCCGTTAACACCT |

Note : [1] Foysal et al. (2019) [2]Haryanti et al. (2017)

Analysis of immune-related genes was performed quantitatively using qPCR and specific primers followed (Foysal et al. 2019) and internal control using lobster actin as presented in Table 11. The qPCR process was conducted using a Thermal

Cycler (Rotor-GeneTM 3000 real-time PCR, USA) with the SensiFAST SYBR® No-ROX kit (Bioline, Australia). The reaction volume for cDNA amplification was 20 µL, consisting of 2x SensiFAST SYBR® No-ROX Mix 10 µL, Forward and Reverse primer pair each 0.8 µL, Nuclease Free Water 15 µL and cDNA sample 5 µL. The cycling temperature conditions for qPCR consisted of denaturation temperature at 95°C (2 minutes) for 1 cycle, followed by 95°C (5 seconds), annealing temperature at 65°C (10 seconds) and extension temperature at 72°C for 20 seconds repeated for 40 cycles. At the end of the PCR process, the cycle threshold of each sample from the tested genes was recorded. The $\Delta^{Ct}$ was calculated against the Ct β-actin (internal control). The value of Ct is calculated from $\Delta^{Ct}$ (tested sample group) - $\Delta^{Ct}$ (initial expression). The representation of different relative multiples to the initial expression was calculated by $2^{-\Delta\Delta Ct}$. To visualize the data, a plot was made from the collected data.

**Statistical analysis**

In this study, only numerical collected data were analysed using SPSS for Windows version 25.0 (IBM, New York, USA). That includes the growth, length increment, final weight, weight gain, proximate composition, amino acids, fatty acids, survival rate, moulting rate, moult interval, mineral contents, and water quality. Prior to the statistical analysis, all data were checked in terms of homogeneity. The effect of the treatments on the physiological response were analysed using an analysis of variance (ANOVA) to see the differences among the treatments. A Turkey HSD were used to find the significance of the means among the data. The significance of the statistical test in this study was evaluated at $P<0.05$.

# CHAPTER 4: Effect of dietary protein to energy ratios on growth, digestive enzyme activity and body composition of captive juvenile spiny lobsters *Panulirus ornatus* (Fabricius, 1798)

## 4.1. Introduction

The *Panulirus ornatus*, commonly known as the tropical or ornate spiny lobster, stands out among commercially exploited lobster species within the Panulirus genus and is notably abundant in the Indo-Pacific region (Holthuis 1991). The commercial fisheries for this species reached a substantial 70,000 tons in 2019 (FAO 2020), reflecting its high demand and value alongside other spiny lobster species. In response to this demand, some countries have shifted to aquaculture-based production of *P. ornatus*, focusing on growing out of the non-feeding stage of spiny lobsters as an initial stock (Jones et al. 2019). This method, recognized as the most efficient for spiny lobster growth, has seen success in countries like Vietnam and Indonesia, with respective total productions of 2,271 tons and 700 tons in 2019 (FAO 2020). As commercially formulated feed for spiny lobsters is not yet available, bivalves are commonly utilized as the natural diet for ornate spiny lobster aquaculture. However, this practice leads to inconsistent production due to inadequate dietary nutrition. Hence, the development of formulated or complete feeds for ornate spiny lobsters is crucial, requiring further research (Phillips and Matsuda 2011; Nankervis and Jones 2022).

Research on the protein requirements for optimizing the growth of *P. ornatus* has yielded varied outcomes (Smith et al. 2003; Smith et al. 2005; Johnston et al. 2008; Irvin and Shanks 2015). While many studies suggest a strong positive correlation between increased dietary protein content and the growth of *P. ornatus* juveniles, recommending high dietary protein levels (Smith et al. 2003; Smith et al. 2005), Irvin and Shanks (2015) found comparable growth responses with lower protein levels in a formulated diet. This indicates that high dietary protein may not be the sole factor maximizing growth in juvenile lobsters. Consequently, it is suggested that the optimum dietary lipid for spiny juvenile lobsters falls within the range of 10-

16% (Johnston et al. 2003; Smith et al. 2003; Smith et al. 2005) surpassing the established lipid requirements for most crustaceans (D'Abramo et al. 1984). Conversely, Irvin and Shanks (2015) found that lower lipid portions (8-9%) in a formulated diet were optimal for spiny lobster growth under cage culture. High dietary lipids are expected to reduce protein requirements or result in better growth through protein sparing, although excessive lipids can lead to fatty acid deposition, reducing growth rates.

Protein plays a significant role in tissue synthesis, but its synthesis occurs only when energy needs are adequately met. Otherwise, protein serves as an energy source (Cuzon and Guillaume 1997). Consequently, investigations into the optimal protein-to-energy (P/E) ratios in a formulated diet become crucial. Few studies on the P/E ratio requirements for ornate spiny juvenile lobsters have been conducted (Smith et al. 2003; Smith et al. 2005). Early nutritional studies on *P. ornatus* juveniles indicated that a high dietary P/E ratio ranging between 28 to 29 mg $KJ^{-1}$ is required for maximum growth and survival (Smith et al. 2003; Smith et al. 2005). However, this study did not delve into the protein and lipid combinations in the protein-energy sparing effect mechanism. It was found that the high lipid content in the formulated diet failed to reduce the protein requirement, and vice versa (Smith et al. 2003). In other crustaceans such as the Chinese mitten crab (*Eriocheir sinensis*), white leg shrimp (*Litopenaeus vannamei*), and oriental river prawn (*Macrobrachium nipponense*), properly formulated dietary protein-to-energy ratios have been proven to maximize growth and immune performance (Cui et al. 2017; Zhang et al. 2017). However, the optimum ratio remains unclear for ornate spiny juvenile lobsters. Therefore, this experiment aims to evaluate the effects of optimal protein in combination with six P/E ratios on the growth, survival, and digestive enzyme activities of *P. ornatus* juveniles.

## 4.2. Materials and Methods

### 4.2.1. Animals

Pueruli weighing 0.25 ± 0.01 g were procured from a local supplier in the Southcoast Province of Banten, Indonesia. These pueruli were introduced into rectangular fiberglass aquaria containing corals, plastic net cuts, ample aeration, and a water filtration system. They were maintained in these conditions for one week until they transformed into pigmented juveniles. Throughout the acclimation period, the pueruli were not provided with any feed, and their overall health was monitored visually. Lobster juveniles displaying signs of poor health, mortality, unattractiveness, or lost body appendages were excluded from the experimental setup.

### 4.2.2. Experiment system and design

In the feeding experiment, the juvenile lobsters were cultured in 18 rectangular 60 L acrylic fish tanks (60 cm × 29.5 cm × 35 cm) for eight weeks. Individual spiny juveniles were placed in 1.5 L plastic baskets with holes and hides to minimize stress, with a total of 8 lobsters per tank. Each tank was equipped with individual water filtration and aeration systems, and the experimental laboratory conditions were maintained under a natural photoperiod (12h day; 12h dark). Tested diets were delivered as moist diets and distributed using stainless steel pincers twice a day. Water quality checks were conducted daily in the morning and evening throughout the entire experimental period using water quality monitoring devices (Horiba U-5000, Japan).

### 4.2.3. Experimental Diet

Six experimental diets containing three protein levels (45%, 50% and 55%), three lipid levels (7%, 9% and 13%) containing digestible energy levels of 19 to 21 g CP $MJ^{-1}$; and six proteins to energy ratios (21, 22, 23, 24, 25 and 26 g CP $MJ^{-1}$), were formulated and assigned as (Basal), high oil (HO), low oil (LO), high, low protein (LP) and low protein-high oil (LP-HO) (Table 12). The fishmeal, obtained from the Fish Nutrition Department at IPB University in Bogor, Indonesia, had an approximate crude protein content of 56%. All feed ingredients, excluding fish oil and soy lecithin, were manually mixed and sieved using a 1.0 mm stainless steel sieve (Retch Test

Sieve, Germany). Following the sieving process, a milling machine (Kawano 250G, Korea) was employed to thoroughly mix all ingredients. The finely formulated mixture was subsequently stored in an airtight plastic bag and kept in a dry location until it was utilized as moist diets.

Table 12. Formulated diet ingredients (dry weight basis) and proximate composition.

| Ingredients | Basal | HO | LO | HP | LP | LP-HO |
|---|---|---|---|---|---|---|
| Fishmeal [1] | 578 | 582 | 540 | 648 | 530 | 530 |
| Astaxanthin (8%)[1] | 2 | 2 | 2 | 2 | 2 | 2 |
| Binder (inert) [1] | 22 | 22 | 22 | 22 | 22 | 22 |
| Casein [2] | 170 | 173 | 191 | 191 | 135 | 147 |
| Wheat (10 CP) [1] | 154 | 107 | 191 | 70 | 233 | 185 |
| Corn/wheat starch[1] | 25 | 25 | 25 | 25 | 25 | 25 |
| Cholesterol[1] | 4 | 4 | 4 | 4 | 4 | 4 |
| Fish oil[1] | 20 | 60 | 0 | 13 | 24 | 60 |
| Vitamin premix[1] | 10 | 10 | 10 | 10 | 10 | 10 |
| Vitamin C[1] | 5 | 5 | 5 | 5 | 5 | 5 |
| Soy lechitin[3] | 5 | 5 | 5 | 5 | 5 | 5 |
| Trace mineral premix[1] | 5 | 5 | 5 | 5 | 5 | 5 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| DM% | 91.33 | 91.79 | 91.06 | 91.5 | 91.15 | 91.58 |
| Ash% | 7.94 | 7.97 | 7.6 | 8.8 | 7.3 | 7.31 |
| GE MJ/kg | 20.4 | 21.35 | 19.86 | 20.73 | 20.04 | 20.93 |
| CP% | 50.22 | 50.24 | 50.19 | 55.28 | 45.18 | 45.74 |
| Lipid% | 9.87 | 13.84 | 7.48 | 9.87 | 9.82 | 13.34 |
| Fibre% | 0.61 | 0.58 | 0.65 | 0.59 | 0.62 | 0.59 |
| **P/E ratio (mg $kJ^{-1}$)** | **24.62** | **23.53** | **25.28** | **26.67** | **22.54** | **21.85** |

**Proximate and biochemical analysis**

The proximate composition of the feed and lobster whole-body were performed according to Chapter 3, section 3.5.2. Biochemical analysis.

### 4.2.4. Water Quality

The water quality measurement was conducted according to Chapter 3, section 3.5.2. Biochemical analysis point (j).

**Growth evaluation**

Physiological data were collected at the beginning, in the middle and at the termination of the feeding trial according to Chapter 3, section 3.5.1. evaluation of growth performance.

### 4.2.5. Statistical analysis

The effects of dietary P/E ratio on all tested parameters of the lobster juvenile were examined using an ANOVA (Chapter 3, section 3.5.6. Statistical analysis). A quadratic regression analysis was performed to analyze the trend of the results.

## 4.3. Results

### 4.3.1. Growth performance

The specific growth rate and length increment were notably influenced ($P<0.05$) by the dietary protein (refer to Table 13). However, the dietary lipid and the interaction between protein and lipid did not yield a significant impact ($P>0.05$) on the specific growth rate and length increments. The survival and moulting rate remained unaffected by the dietary protein, lipid, and the interaction between protein and lipid levels. While the moult interval was not influenced by any P/E ratio, it exhibited a significant response to the dietary lipid level.

Table 13. The physiological response of lobster juveniles fed various dietary P/E ratios.

| Diets | SGR (% g.day$^{-1}$) | Survival rate (%) | Increment of length (%) | Moulting rate (%) | MI (days) |
|---|---|---|---|---|---|
| Basic | 0.36 ± 0.06 $^{b}$ | 58.3 ± 11.0 | 7.56 ± 1.34 | 62.50 ± 19.09 | 20.0 ± 1.0 |
| HO | 0.47 ± 0.07 $^{a,b}$ | 70.8 ± 11.0 | 5.94 ± 1.64 | 45.83 ± 8.33 | 14.5 ± 7.8 |
| LO | 0.53 ± 0.08 $^{a,b}$ | 58.3 ± 8.33 | 12.13 ± 2.79 | 83.33 ± 11.02 | 23.8 ± 6.1 |
| HP | 0.69 ± 0.05$^{a}$ | 58.3 ± 11.0 | 13.20 ± 3.84 | 83.33 ± 11.02 | 25.2 ± 5.3 |
| LP | 0.36 ± 0.10 $^{b}$ | 54.1 ± 11.0 | 1.69 ± 2.89 | 83.33 ± 16.67 | 28.3 ± 10.5 |
| LP-HO | 0.58 ± 0.04 $^{a,b}$ | 58.3 ± 4.43 | 7.49 ± 1.95 | 62.50 ± 14.43 | 18.3 ± 3.5 |

Values are presented as means ± SE from three replicates. Data in the similar column with different superscripts ($^{a,b}$) indicated a significant difference in means value at $P<0.05$.

The survival rate of lobster juveniles decreases gradually from the first week to the completion date with a similar final survival rate in all treatments (

Figure 3). Polynomial regression analysis indicated the relationship between the P/E ratio (x) and specific growth rate as $Y= 0.0375x^2 – 1.7347x + 20.439$, with $R^2 = 0.8036$ (Figure 4)

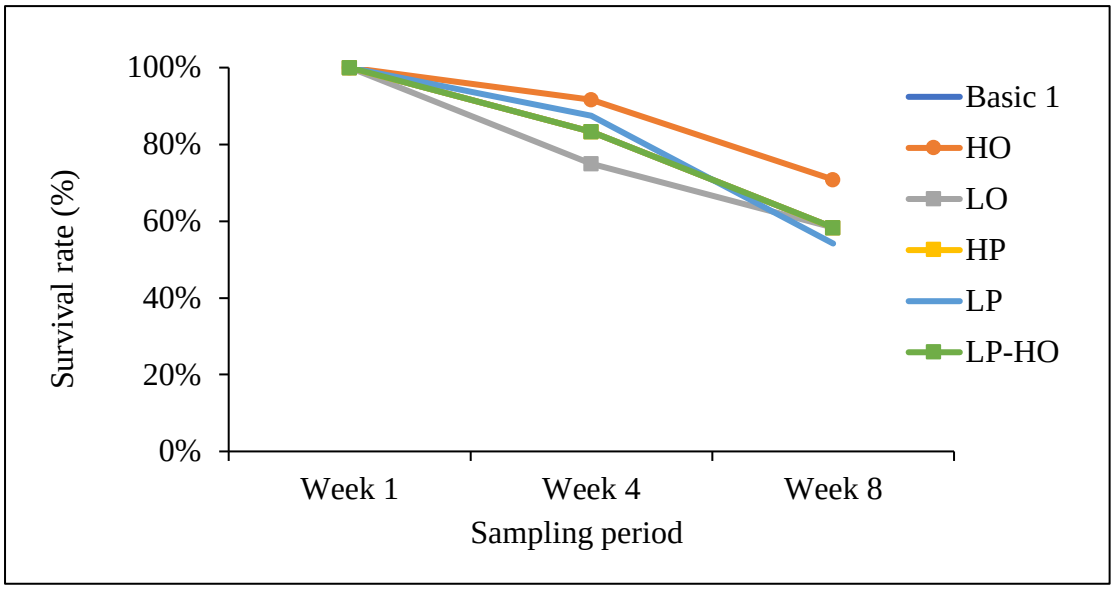



Figure 3. The survival rate of juvenile lobster fed different dietary P/E ratios

### 4.3.2. Digestive enzyme

Protease and lipase increased in juvenile lobsters fed a high P/E ratio ($P<0.05$). Amylase was not significantly affected by the ratios. Superoxidase dismutase was significantly affected by the treatment. Dietary protein, lipid and their interaction affect the protease, lipase, and SOD significantly. The interaction between the dietary protein and lipid did not show a significant effect on SOD. The protease, lipase, amylase and superoxidase dismutase results is presented in Table 14.

Table 14. Digestive enzyme activity and super oxidase dismutase of early spiny lobster juvenile following dietary of different P/E ratio.

| Diets (P/E ratio) | Protease | Lipase | Amylase | SOD |
|---|---|---|---|---|
| Basic | 18.91 ± 1.4 [b] | 11.62 ± 1.16[b] | 1.78 ±0.06 | 8.60 ± 0.32[b] |
| HO | 18.26 ± 0.6 [a] | 22.09 ± 1.16[c] | 1.42 ± 0.02 | 10.26 ± 0.12[c] |
| LO | 19.20 ± 1.7[b] | 5.81 ± 0.58[a] | 2.05 ± 0.08 | 6.26 ± 0.19[a] |
| HP | 21.14 ± 0.3 [d] | 28.48 ± 0.58[d] | 1.52 ± 0.02 | 7.39 ± 0.51[a, b] |
| LP | 18.81 ± 0.4 [a, b] | 18.60 ± 1.16[c] | 1.81 ± 0.03 | 7.97 ± 0.20[b] |
| LP-HO | 19.58 ± 0.1 [c] | 4.65 ± 1.16[a] | 2.13 ± 0.38 | 10.84 ± 0.17[c] |

Values are presented as means ± SE from three replicates. Data in the similar column with different superscripts ([a, b]) indicated a significant difference in means value at $P<0.05$.

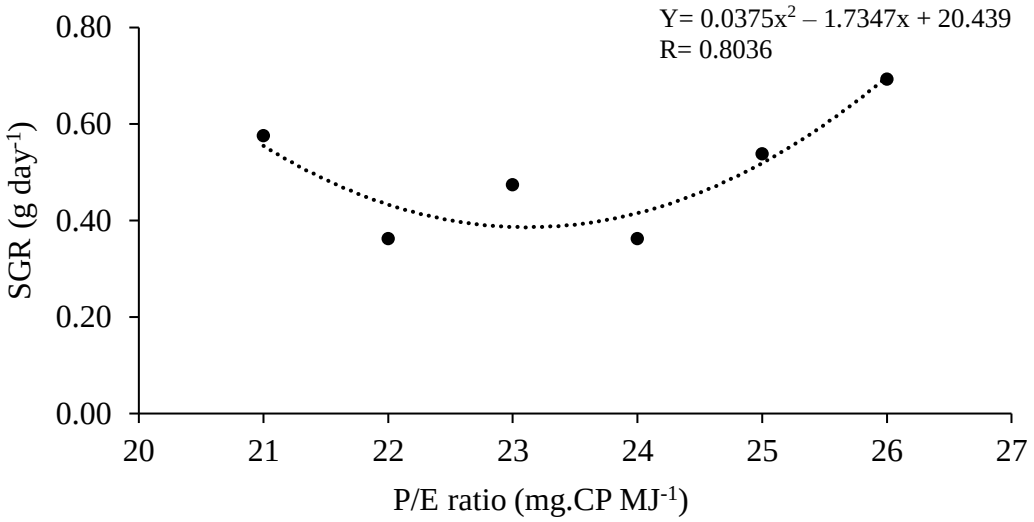



Figure 4. The specific growth rate of juvenile *P. ornatus* fed different dietary P/E ratios in an 8-week feeding trial.

### 4.3.3. Tissue Proximate composition

The treatments had a significant impact ($P<0.05$) on the levels of protein, lipid, ash, crude fiber, and NFE (Table 15). The interaction between dietary protein and lipids exerted a noteworthy influence on the protein and lipid content in the entire

Table 15. The whole-body proximate composition of spiny lobster juveniles fed different dietary P/E ratios. Values are presented as means ± SE from three replicates. Data in the similar column with different superscripts ($^{a,b}$) indicated a significant difference in means value at P<0.05.

| Diets | Protein | Lipid | Ash | Crude fiber |
|---|---|---|---|---|
| Basic | 20.99 ± 1.19 $^{a}$ | 6.07 ± 0.12 $^{c,d}$ | 58.39 ± 0.67$^{a,b}$ | 6.24 ± 0.28 $^{b}$ |
| HO | 30.27 ± 0.38 $^{b}$ | 7.19 ± 0.24 $^{e,f}$ | 62.39 ± 1.45 $^{b,c}$ | 4.89 ± 0.12 $^{a,b}$ |
| LO | 23.05 ± 0.15 $^{a}$ | 5.76 ± 0.19 $^{b,c}$ | 56.94 ± 0.25 $^{a}$ | 4.81 ± 0.27 $^{a,b}$ |
| HP | 33.86 ± 0.21 $^{c}$ | 4.82 ± 0.29 $^{a,b}$ | 63.34 ± 0.47 $^{c}$ | 4.27 ± 0.04 $^{a,b}$ |
| LP | 19.90 ± 0.79 $^{a}$ | 3.99 ± 0.12 $^{a}$ | 58.59 ± 0.62 $^{a,b}$ | 4.64 ± 0.29 $^{a,b}$ |
| LP-HO | 28.29 ± 0.13 $^{b}$ | 7.05 ± 0.13 $^{d,e}$ | 56.60 ± 0.21 $^{a}$ | 3.07 ± 0.31 $^{a}$ |

Table 16. Amino acid analysis profile of whole-body lobster juvenile (%)

| | Basic | HO | LO | HP | LP | LP-HO |
|---|---|---|---|---|---|---|
| **Non-Essential amino acids** | | | | | | |
| Alanine | 1.57 | 1.50 | 1.42 | 1.52 | 1.37 | 1.44 |
| Aspartate | 1.77 | 1.70 | 1.57 | 1.62 | 1.59 | 1.65 |
| Glutamine | 3.31 | 3.19 | 2.97 | 3.01 | 2.89 | 2.99 |
| Serine | 0.65 | 0.68 | 0.59 | 0.65 | 0.62 | 0.58 |
| Glycine | 1.39 | 1.29 | 1.21 | 1.20 | 1.18 | 1.21 |
| Tyrosine | 0.69 | 0.63 | 0.65 | 0.57 | 0.61 | 0.64 |
| Proline | 0.42 | 0.45 | 0.41 | 0.44 | 0.39 | 0.41 |
| Total | 9.80 | 9.43 | 8.83 | 9.00 | 8.65 | 8.90 |
| *SSd* | 0.00 | 0.04 | 0.22 | 0.16 | 0.01 | 0.18 |
| **Essential amino acids** | | | | | | |
| Histidine | 0.63 | 0.68 | 0.59 | 0.61 | 0.59 | 0.55 |
| Arginine | 0.51 | 0.47 | 0.47 | 0.47 | 0.48 | 0.43 |
| Threonine | 0.99 | 0.94 | 0.90 | 0.91 | 0.93 | 0.93 |
| Valine | 1.11 | 1.17 | 1.08 | 1.12 | 1.05 | 0.99 |
| Methionine | 0.40 | 0.41 | 0.37 | 0.40 | 0.37 | 0.38 |
| Cysteine | 0.33 | 0.31 | 0.34 | 0.33 | 0.31 | 0.31 |
| Isoleucine | 0.42 | 0.42 | 0.45 | 0.47 | 0.42 | 0.47 |
| Leucine | 0.82 | 0.80 | 0.81 | 0.79 | 0.81 | 0.79 |
| Phenylalanine | 0.48 | 0.46 | 0.44 | 0.47 | 0.46 | 0.42 |
| Lysine | 0.78 | 0.75 | 0.72 | 0.74 | 0.76 | 0.71 |
| Total | 6.46 | 6.40 | 6.16 | 6.30 | 6.18 | 5.98 |

bodies of juvenile lobsters. Elevated lipid levels in the diet were found to correlate with increased lipid content in the juveniles' entire bodies. A consistent trend was observed in the proximate composition, indicating that higher dietary lipid and protein contents were reflected in the overall composition of juveniles' bodies.

### 4.3.4. Amino Acids

In both the dietary composition and the entire body of juvenile lobsters, non-essential amino acids are predominant compared to essential amino acids (Table 16 and Table 17, respectively).

Table 17. Amino acid analysis profile of tested diet (%)

| Non-Essential amino acids | Diet types | | | | | |
|---|---|---|---|---|---|---|
| | Basic | HO | LO | HP | LP | LP-HO |
| Alanine | 1.67 | 1.58 | 1.58 | 1.68 | 1.50 | 1.48 |
| Aspartate | 1.82 | 1.77 | 1.77 | 1.79 | 1.69 | 1.72 |
| Glutamine | 3.47 | 3.67 | 3.21 | 3.51 | 3.18 | 3.24 |
| Serine | 0.70 | 0.72 | 0.65 | 0.71 | 0.70 | 0.68 |
| Glycine | 1.42 | 1.35 | 1.34 | 1.38 | 1.37 | 1.40 |
| Tyrosine | 0.79 | 0.69 | 0.79 | 0.69 | 0.79 | 0.87 |
| Proline | 0.51 | 0.53 | 0.47 | 0.57 | 0.50 | 0.52 |
| Total | 10.37 | 10.31 | 9.81 | 10.33 | 9.74 | 9.89 |
| *SSd* | 0.00 | 0.07 | 0.09 | 0.02 | 0.02 | 0.10 |
| Essential amino acids | | | | | | |
| Histidine | 0.69 | 0.79 | 0.68 | 0.79 | 0.70 | 0.69 |
| Arginine | 0.57 | 0.51 | 0.58 | 0.59 | 0.59 | 0.60 |
| Threonine | 1.12 | 1.15 | 0.98 | 1.12 | 1.15 | 1.01 |
| Valine | 1.43 | 1.33 | 1.28 | 1.28 | 1.25 | 1.17 |
| Methionine | 0.46 | 0.51 | 0.49 | 0.56 | 0.48 | 0.50 |
| Cysteine | 0.39 | 0.40 | 0.50 | 0.47 | 0.47 | 0.50 |
| Isoleucine | 0.50 | 0.51 | 0.59 | 0.61 | 0.62 | 0.66 |
| Leucine | 0.90 | 0.89 | 0.89 | 0.97 | 0.98 | 0.96 |
| Phenylalanine | 0.59 | 0.57 | 0.62 | 0.60 | 0.58 | 0.54 |
| Lysine | 0.89 | 0.84 | 0.81 | 0.89 | 0.87 | 0.83 |
| Total | 7.52 | 7.50 | 7.42 | 7.89 | 7.66 | 7.45 |
| *SSd* | 0.00 | 0.03 | 0.07 | 0.07 | 0.04 | 0.12 |

The elevated protein content in the tested diets led to a greater quantity of amino acids, both essential and non-essential, compared to diets with lower protein levels. The basal diet, characterized by higher levels of both EAA and NEAA, contributed to the overall amino acid composition in the bodies of juvenile lobsters.

## 4.4. Discussion

The dietary P/E ratio did not affect the growth, survival, and moulting rate of spiny lobster juveniles statistically. However, consistent patterns of growth, total length increment, intermolt period, moulting rate and survival rate were achieved by lobster fed with a high P/E ratio of 26.6 mg $MJ^{-1}$. Several studies indicated that the optimum P/E ratio plays an important role in spiny lobster juvenile growth (Glencross et al. 2001; Smith et al. 2003; Ward, Carter, Crear, et al. 2003). In *P. cygnus* juvenile, the dietary P/E ratio of 27.6 mg $MJ^{-1}$ was observed as the optimum for growth (Glencross et al. 2001). Meanwhile, *J. edwardsii* and *P. ornatus* required a higher P/E ratio between 29 - 30 mg $MJ^{-1}$. Although the lipid content does not affect all of the physiological parameters, the protein content in the diet has resulted in a positive interaction with the specific growth rate, carapace length increment and the 50% success moulting rate (Table 13). In the present study, the specific growth rate achieved by lobster juveniles was low (0.36 to 0.69 % g $day^{-1}$) and comparable to previous studies on spiny lobster juveniles (Smith et al. 2003; Jones and Shanks 2009; Huu and Jones 2014; Kurnia et al. 2017). In contrast, recent nutritional research on the same species conducted by Marchese et al. (2018) reveals that spiny lobster juveniles with an average weight of 1.98 g resulted in higher weight gain. Haryanti et al. (2017) also reported the final growth of *P. homarus* juvenile reared in the floating cage was higher (2.9 to 3.4 g) following three months of feeding trial. The low growth rate of spiny lobster juveniles in the present study can be attributed to the initial animal size and container size used in the feeding trial. The spiny lobster juvenile used in the present study was very small, only 0.3 g on average and the same size as the lobster juvenile used by Kurnia et al. (2017). Meanwhile, a larger lobster juvenile was used in two studies reported (Haryanti et al. 2017; Marchese et al. 2018). Different initial size of lobster was reported to have different growth rates under a

controlled culture system (Jones and Shanks 2009). There was also an effect on the size of individual cages to the growth of spiny lobster. In a feeding trial conducted by Haryanti et al. (2017), larger cages were used which resulted in a higher specific growth rate. Despite the inconsistent results of growth, the association of P/E ratio to specific growth rate in the present study was the same as initially reported (Smith et al. 2003; Smith et al. 2005). At the end of the week's feeding trial, the survival rate of spiny lobster juveniles ranges from 54.1 to 70.8 %. This survival rate is high and in agreement with previous research on spiny lobsters (Haryanti et al. 2017; Kurnia et al. 2017; Marchese et al. 2018). The individual culture system seem to have a critical function to protect the spiny lobster juvenile from canibalism (Haryanti et al. 2017; Marchese et al. 2018). In the present study, the spiny lobster were dead mostly during the moulting process because of unable to moult completely which may attributed to the lack of the nutrition for the ecdysis process as previously reported (Irvin and Williams 2009a).

Moulting or ecdysis is a sign of the growth in crustaceans as the result of the development of the exoskeleton. In crustaceans including spiny lobsters, a moult is an important event of growth that requires much energy during the process. The increase of moulting and growth rate of spiny lobster has been associated with the rearing system, size and feed type (Irvin and Williams 2009a; Jones and Shanks 2009; Vijayakumaran et al. 2010) and lack of information about this event following the P/E ratio treatment. In the present study, the final moulting rate of spiny lobster ranges between 45.83 to 83.33 %, with an intermolt period of 23 to 44 days. It is difficult to associate both the moulting rate and intermolt period directly with the P/E ratio treatment. However, results indicated that the diet with a high P/E ratio (medium lipid content) resulted in a high moulting rate and intermolt period (Table 13). On the contrary, higher lipid content exhibits a lower moulting rate and also extended intermolt period of spiny lobster juveniles. The phospholipid and sterol were found to have an important role during the moulting process of crustacea (Teshima 1998) and excessive lipid content within the diet has a detrimental effect on the growth of crustaceans. The moderate lipid level in the present study (9%) seems to be optimal

for *P. ornatus* and was similar to the previous reports (Glencross et al. 2001; Smith et al. 2003). In addition, the communal rearing system was found to have significant results in the moulting rate in *P. ornatus* (Vijayakumaran et al. 2010) and supported by a recent study (Marchese et al. 2018). Starve condition was found to prolong the intermolt period of *P. argus* (Espinosa-Magaña et al. 2017). Marchese et al. (2018) reported that the spiny lobster juvenile reared communally resulted in more frequent moulting compared to the individual rearing system. They also found that the number of moults was greater on the lobster fed with fresh feed compared to the formulated diet.

The activity of digestive enzymes including protease, lipase and carbohydrase in spiny lobster have been evaluated in the larval phase of *P. ornatus* (Johnston et al. 2003; Perera et al. 2008; Simon 2009; Rodriguez-Viera et al. 2016; Rodríguez-Viera et al. 2017; Méndez-Martínez et al. 2018). Further, some studies have successfully identified the role of those digestive enzymes on spiny lobster species (Johnston 2003; Johnston, Aj, et al. 2004). The digestive enzyme of spiny lobster was reported to be associated with the size or larval stage (Johnston 2003; Johnston, Aj, et al. 2004; Johnston, Ritar, et al. 2004), moult event (Perera et al. 2008), diet type (Hung et al. 2015), water quality (Hung et al. 2015) and disease infection (Herrera-Salvatierra et al. 2019). Nonetheless, the P/E ratio has been reported to have a significant effect on digestive enzyme activity in other crustacean species (Cui et al. 2017; Méndez-Martínez et al. 2018; Sudtongkong et al. 2020). In the present study, the digestive enzyme of juvenile spiny lobster except total amylase was affected by the dietary P/E ratio of the feed. Both lipase and protease enzymes were significantly influenced by the level of protein and lipid, as well as the interaction of both of them. In spiny lobster *J. edwardsii*, the interaction of some digestive enzymes was well documented and it was found that the enzyme activity was found to have a strong relationship with the stage of the species (Simon 2009). The effect of dietary types on the digestive enzyme of the juvenile spiny lobster, *J. edwardsii* has been also previously reported (Simon 2009). Artificial feed regimes in that feeding trial resulted in significantly different amylase and total protease activity. In addition, Cui et al. (2017) reported a

similar pattern of interaction between protease activity and the increase of dietary protein in Chinese mitten-handed crab (*E. sinensis*).

The total amylase activity of lobster in the present study is not significantly different. However, the enzyme activity was found to have a strong relationship with the stage of spiny lobster, *J. edwardsii*. In addition, low-level digestive enzymes were also available in the non-feeding stage of spiny lobster (Johnston 2003). Previous studies indicated that the increase in dietary level of protein improves the amylase activity in crustaceans (Méndez-Martínez et al. 2018; Sudtongkong et al. 2020). In contrast, *E. singaporense* exhibits a decrease in amylase enzyme activity with the increase of the dietary protein in the diet (Sudtongkong et al. 2020). Apart from nutritional changes, the disease infection also influences the amylase enzyme activity (Herrera-Salvatierra et al. 2019). Although the amylase enzyme activity was not significantly different, spiny lobster juveniles fed lower dietary protein sources as well as low P/E ratio in the present study performed on the higher amylase enzyme activity.

The optimum P/E ratio for spiny lobster was reported by Smith et al. (2003) to be 29.2 mg $MJ^{-1}$ at 530 and 100 g $kg^{-1}$ of protein and lipid respectively. Smith et al. (2005) concluded that high crude protein in the diet with a higher P/E ratio (mg $MJ^{-1}$) of formulated diet is optimal for the growth of *P. ornatus* juvenile. Ward, Carter, Crear, et al. (2003) found that the formulated diet containing a P/E ratio of 29 mg $MJ^{-1}$ is suitable for *J. edwardsii* to achieve optimum growth. They conclude that the growth, survival, and body composition of spiny lobsters were highly influenced by the level of P/E ratio of the diet. Tissue proximate analysis indicated that the dietary P/E ratio significantly affects the protein, lipid, ash, crude fiber and NFE. In the present study, the P/E ratio of 21 mg $MJ^{-1}$ resulted in the highest crude protein level of spiny lobster whole-body tissue. In contrast, the value of lipid, ash, crude fiber and NFE of the lobster juvenile were significantly low following this dietary P/E ratio. Higher protein content within the body was also found in lobster juveniles fed a dietary P/E ratio of 23 mg $MJ^{-1}$. Both dietary have a high content of lipid (13L) and low protein content. In contrast, the protein content in body composition of freshwater

crustacean *M. niponense* was not affected by the dietary protein content in the diets (Zhang et al. 2017). It also occurred in freshwater lobster *P. clarkii* (Xu et al. 2013) and Chinese mitten crab (Cui et al. 2017).

## 4.5. Summary

Current research reveals that elevating the dietary protein-to-energy (P/E) ratio enhances the growth performance and digestive enzyme activities of juvenile lobsters. Moreover, even with a P/E ratio lower than the recommended value, the growth and survival rates of juvenile lobsters in this study fall within an acceptable range. Evaluating growth rate, total length increment, and the activities of protease and lipase enzymes, the optimal diet for *P. ornatus* juveniles consists of 55% protein and 9% lipid, resulting in a P/E ratio of 26.6 mg $KJ^{-1}$. Conversely, juvenile lobsters experience suboptimal growth at a P/E ratio of 23.12 mg $KJ^{-1}$. Given the increasing focus on feed development for spiny lobster aquaculture, there is a growing demand for further exploration into the use of alternative ingredients beyond fishmeal for the nutrition of juvenile lobsters.

# CHAPTER 5. Nutritive composition of commercial full fat and defatted black soldier fly larvae meal (*Hermetia illucens*) as a potential protein source for aquafeeds

## 5.1. Introduction

In recent times, significant attention has been directed towards the extensive exploration of the black soldier fly as a sustainable future nutrition source. Despite its recognition as a nutritious food option for human consumption (Bessa et al. 2020), it is imperative to delve further into both its positive and negative effects (Bessa et al. 2021). The black soldier fly has emerged as an optimal nutritional resource for animal feed, playing crucial roles in agriculture such as providing natural fertilizers, feed and oil for livestock, chitin sources (Soetemans L et al. 2020), and serving as a reservoir for antimicrobial peptides (Xia et al. 2021). Advancements in technology have revealed the potential of the black soldier fly in supplying protein for animal feed, particularly as aquafeeds for fish and shrimp (Riddick 2014; Henry et al. 2015). This is attributed to the comparable nutritional content of black soldier fly to fishmeal, proving sufficient to promote the growth of fish and shrimp. Beyond being an alternative protein source in fish feed, the black soldier fly is also being considered for its potential to supply lipids (Franco et al. 2021). Various end products derived from the black soldier fly include, but are not restricted to, live specimens, oven-dried variants, black soldier fly larvae (BSFL) meal powder, and frass (Yildirim-Aksoy et al. 2020; Zulkifli et al. 2022).

BSF larvae encompass a diverse range of nutrients, including protein, lipid, minerals, vitamins, amino acids, and fatty acids. Numerous studies have indicated that the protein content in BSF varies between 30-40%, contingent on factors such as stage, substrate, and processing. This variability underscores its potential as a substitute for fishmeal in aquafeeds, supporting the evolution of aquaculture (Devic et al. 2018; Fisher et al. 2020; Hu et al. 2020; Zozo et al. 2022; Zulkifli et al. 2022). The protein levels in BSF are well-suited for serving as protein sources in the diets of cultured fish and crustaceans with moderate crude protein requirements. Enhancing

the nutritional quality of BSF is a pertinent objective. Reports indicate that BSF larvae have a high lipid content, raising concerns about their use as alternative protein sources in aquafeeds. Addressing this concern involves reducing excessive lipid content through a defatting process. By using a solution of hexane and isopropanol, the defatting process has proven effective in removing approximately 80% of the total fat content from BSF larvae, consequently increasing the crude protein content by about 22.4% (Zozo et al. 2022). This elevation in crude protein content also augments the availability of amino acids. Schiavone et al. (2017) observed that all amino acids in highly-defatted BSF exceeded those in partially-defatted BSF, influencing the amino acid composition of the tested diets.

Besides the successful replacement of fishmeal with full-fat BSF larvae, it has been documented that defatted BSF larvae can also serve as fishmeal substitutes in the diets of various fish and crustaceans, yielding mixed results (Li et al. 2017; Hu et al. 2020; Hender et al. 2021). Studies indicate that the crude fat content of full-fat BSF larvae can reach up to 32% (Hu et al. 2020; Weththasinghe et al. 2021). This nutritious lipid is considered a sustainable source for future aquafeeds, primarily due to its straightforward production (Ewald et al. 2020; Franco et al. 2021). Similar to protein content, the lipid content in BSF larvae significantly varies depending on the substrate or food source (Barragan-Fonseca et al. 2017). Additionally, the stage and age of the larvae are crucial factors influencing the nutritional composition of BSF. BSF raised on household waste substrates are known to exhibit high fat content, whereas those reared on fruit and vegetable substrates have lower protein content compared to those cultivated using animal materials. Nguyen Quang et al. (2015) reported that the protein content of BSF grown on fruit and vegetable substrates had a crude protein value 38.5% lower than BSF cultivated using fish or ruminant liver substrates.

Both full-fat and defatted BSF have been recognized for their potential role as substitutes for fishmeal. Numerous scholarly works delve into the nutritional aspects of BSF, highlighting it as a sustainable and promising future source of nutrition (Rabani et al. 2019; Xu et al. 2020; Hender et al. 2021; Weththasinghe et al. 2021; Lu

et al. 2022). Each type of BSF meal possesses distinct characteristics, and both have pros and cons in terms of their effects on the physiology and immunology of cultured animals. Consequently, understanding the nutritional content of BSF becomes crucial for obtaining accurate information, especially for commercially available BSF products in the market. This study aims to compare commercial full-fat BSF and defatted BSF meal, both accessible in the local market, serving as alternative protein sources in aquafeeds. By elucidating the nutritional content of BSF larvae meal, precise information can be obtained, facilitating the formulation of fish feed that aligns with the specific nutritional needs of various fish species.

## 5.2. Material and Methods

In the present study, commercial BSF larvae were purchased from marketplace, locally produced in Indonesia. The defatted BSF was purchased in form of meal while the full fat BSF was in oven-dried form. The full fat oven-dried BSF larvae were then grinded using food processor (Chence CH-250A, China) several times until fine particles are achieved. The product was also sieved using industrial sieve (Retsch Test Sieves, US). Both BSF meal were sent to the laboratory for further analysis including proximate composition, amino acids, fatty acids, and minerals.

### 5.2.1. Proximate composition

The proximate composition of full-fat and defatted BSF were analysed using method as described in Chapter 3, section 3.5.2. Biochemical analysis.

### 5.2.2. Amino Acids

The amino acids analysis was determined using the procedure in Chapter 3, section 3.5.2. Biochemical analysis, point (f).

### 5.2.3. Fatty acids

The amino acids analysis was determined using the procedure in Chapter 3, section 3.5.2. Biochemical analysis, point (g).

### 5.2.4. Mineral content

The amino acids analysis was determined using the procedure in Chapter 3, section 3.5.2. Biochemical analysis, point (h).

### 5.2.5. Percent difference

The amino acids analysis was determined using the procedure in Chapter 3, section 3.5.2. Biochemical analysis, point (i).

## 5.3. Result

The analysed proximate composition of the tested BSF meal revealed that defatted BSFL exhibited elevated levels of crude protein and ash compared to full-fat BSFL. In contrast, the defatted BSFL had lower crude lipid content than the full-fat BSFL meal, as outlined in (Table 18). Additionally, the moisture content of full-fat BSFL was relatively similar to the ash content in the defatted BSFL meal.

Table 18. The proximate composition of commercial full fat and defatted BSF meal

| Proximate composition (%) | FF BSFL | DF BSFL | % differences |
|---|---|---|---|
| Crude protein | 30.72 | 47.70 | 55.3 |
| Crude Lipid | 36.20 | 8.11 | -77.6 |
| Ash | 11.97 | 18.90 | 57.9 |
| Moisture | 6.56 | 6.02 | -8.2 |

In general, the content of amino acids in the full fat BSFL was higher than the amino acids in defatted BSF. Exception for threonine and histidine where their content in the full fat BSF is lower (Table 19). The comparative analysis of the fatty acid composition of the tested samples is dislayed in Table 20. In defatted BSFL meal, all tested fatty acid contents were higher than those in full-fat BSFL meal. Notably, both capric (C10:0) and caprylic (C8:8) were undetectable in both types of BSFL meals. Arachidic acid (C20:4 n6) was present in defatted BSFL meal but absent in full-fat BSFL meal. Significant amounts of palmitic acid (C16:0), oleic acid (C18:1 n9), and linoleic acid (C18:2) were observed in both types of BSFL meals. The most substantial differences in fatty acid content were observed in lauric acid, with variations exceeding 80%. Additionally, arachidic acid was absent in defatted BSFL, whereas only a small quantity was found in full-fat BSFL.

Table 19. Amino acids composition of commercial full fat and defatted BSF

| Amino acid | DF BSFL[a] | FF BSFL | Differences (%) |
|---|---|---|---|
| Aspartate | 1.84 | 2.35 | -21.7 |
| Glutamine | 3.12 | 3.21 | -2.8 |
| Serine | 0.71 | 1.65 | -57.0 |
| Glycine | 0.84 | 1.85 | -54.6 |
| Histidine | 0.72 | 0.43 | 67.4 |
| Arginine | 0.70 | 1.23 | -43.1 |
| Threonine | 0.82 | 0.77 | 6.5 |
| Alanine | 0.72 | 1.28 | -43.8 |
| Proline | 0.84 | 1.82 | -53.8 |
| Tyrosine | 0.71 | 0.78 | -9.0 |
| Valine | 0.61 | 1.27 | -52.0 |
| Methionine | 0.52 | 0.61 | -14.8 |
| Cysteine | 0.62 | 0.74 | -16.2 |
| Isoleucine | 0.88 | 1.18 | -25.4 |
| Leucine | 1.30 | 2.13 | -39.0 |
| Phenylalanine | 0.69 | 0.88 | -21.6 |
| Lysine | 1.26 | 2.16 | -41.7 |

[a] Amino acid profile of defatted BSFL from Saputra and Fotedar (2023)

The minerals analysis from the full fat and defatted BSF indicated that defatted BSF has higher amount of all mineral type tested than full fat BSF. The complete mineral analysis results can be seen in Table 21. Calcium is the largest proportion of mineral from both BSFL meal followed by magnesium and natrium, respectively. In contrast, only small amount of copper was found in both BSFL meal. The copper and zinc were the minerals which have a noticeably differences.

Table 20. Fatty acids composition of commercial full fat and defatted BSFL

| Fatty acids | DF BSFL | FF BSFL | Differences (%) |
|---|---|---|---|
| Caprylic acid (C8:0) | - | - | - |
| Capric acid (C10:0) | - | - | - |
| Lauric acid (C12:0) | 1.23 | 7.21 | -82.9 |
| Myristic acid (C14:0) | 7.52 | 10.23 | -26.5 |
| Palmitic acid (C16:0) | 15.23 | 26.45 | -42.4 |
| Stearic acid (C18:0) | 1.85 | 4.12 | -55.1 |
| Arachidic acid (C20:4 n6) | - | 0.23 | 0.0 |
| Oleic acid (C18:1 n9) | 17.12 | 23.45 | -27.0 |
| Linoleic acid (C18:2) | 26.12 | 34.32 | -23.9 |
| Linolenic acid (C18:3 n3) | 1.05 | 2.12 | -50.5 |
| ∑ PUFA** | 1.05 | 2.35 | -55.3 |
| ∑ MUFA* | 18.17 | 25.57 | -28.9 |
| ∑ SFA*** | 25.83 | 48.01 | -46.2 |

- = not detected

Table 21. Mineral composition of commercial full fat and defatted BSFL

| Minerals | DF BSFL | FF BSFL | Differences (%) |
|---|---|---|---|
| Calcium | 57.7 | 37.0 | 55.9 |
| Natrium | 1.7 | 1.3 | 30.8 |
| Magnesium | 6.2 | 4.2 | 47.6 |
| Zinc | 138.08 | 61.71 | 123.8 |
| Iron | 529.57 | 341.35 | 55.1 |
| Manganese | 128.26 | 90.28 | 42.1 |
| Copper | 26.6 | 5.61 | 374.2 |

*The value for calcium, natrium & magnesium are presented as $g.kg^{-1}$. The value of zinc, iron, manganese and copper are presented as $mg.kg^{-1}$.

### 5.4. Discussion

The demand for the potential use of black soldier fly larvae (BSFL) meal as a protein source in aquafeeds has prompted the evaluation of the nutritional

composition of commercially available BSFL meal. The nutritional composition of both full-fat and defatted BSFL meals varied, aligning with findings from previous studies (Cummins et al. 2017; Biasato et al. 2019; Fisher et al. 2020; Zozo et al. 2022). The defatting process, aimed at reducing fat content, resulted in an increase in the crude protein content of BSFL meal. Zozo et al. (2022) noted a substantial reduction in fat content through the defatting process. Specifically, the fat content of defatted BSFL was found to be 81.11% lower (recalculated) than that of full-fat BSFL, indicating the positive impact of the defatting process on BSFL meal quality, particularly in terms of fat content. Conversely, this process enhanced the crude protein content by up to 19.6%, with the defatted BSFL meal containing 56.11% crude protein of the total dry matter. In a previous study by Biasato et al. (2019), defatted BSF meal was included as a protein resource with a crude protein content of 55.9%, supporting the results of the current study. Several earlier reports, using what appears to be full-fat BSF meal in their experiments, demonstrated crude protein content ranging between 32.1% to 48.2% (Devic et al. 2018; Adeoye et al. 2020; Hu et al. 2020; Rawski et al. 2020; Weththasinghe et al. 2021; Zulkifli et al. 2022) (Table 22). This suggests that full-fat BSF meal may only have a crude protein content of up to 50% of the total dry matter without the defatting process and vice versa. In addition to enhancing crude protein content, the defatting process significantly reduces the fat content in BSF meal. Excessive lipid in aquafeed ingredients can be a limiting factor for certain fish and crustaceans, leading to suboptimal growth and an increased risk of disease in aquaculture. While the lipid content of full-fat BSFL meal can reach as high as 30% of the total dry matter (Hu et al. 2020; Rawski et al. 2020; Weththasinghe et al. 2021), the defatting process reduces the fat content to only 10% of the total dry matter (Zozo et al. 2022). This explains the results of the current study, where the fat content of defatted BSFL meal was only 9% of the total dry matter, lower than that of full-fat BSFL meal.

The defatting process of black soldier fly larvae has been noted to influence major nutritional compositions, as reported by previous studies *(Schiavone et al. 2017; Hender et al. 2021; Zozo et al. 2022)*. In the current study, the amino acid

content of defatted BSFL meal consistently surpasses that of full-fat BSFL meal, potentially influenced by the defatting process. Numerous studies have reported varying results in amino acid analyses of BSF, particularly in terms of dispensable amino acids. For instance, alanine ranged from 1.3 to 7.7, aspartate from 1.7 to 9.3, cysteine from 0.1 to 2.4, glutamine from 3.4 to 11.6, glycine from 0.2 to 3.9, proline from 1.1 to 5.9, serine from 1.2 to 7.1, and tyrosine from 0.8 to 5.8 *(*Table *23)*.

Table 22. Proximate composition of BSF from different sources

| Proximate composition | References | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1)¶ | (2)¶ | (3)¶ | (4)* | (5)¶ | (6)* | (7)¶ | (8)¶ | (9)† | (10)¶ | (11)† |
| Protein | 48.2 | 32.1 | 42 | 56 | 41.6 | 52 | 42.6 | 35 | 55.9 | 45.8 | 56.1 |
| Lipid | 25.7 | 32.2 | 32 | 11.8 | 23.2 | 15.1 | 23 | 29.8 | 8.5* | 25.8 | 4.8 |
| Fibre | 9.9 | NA | NA | NA | 7.6 | NA | 10.8 | 7.9 | NA | NA | NA |
| Moisture | 7.1 | 8.5 | NA | NA | NA | 10 | 8.2 | NA | NA | 4.4 | 6.5 |
| Ash | 8.3 | 13 | 9.25 | NA | 11.6 | 7.3 | 10.5 | 5.3 | 7.6 | NA | NA |
| NFE | 7.9 | NA | NA | NA | 10.8 | NA | 4.9 | 22.1 | NA | NA | NA |

(1) Zulkifli et al. (2022); (2) Hu et al. (2020); (3) Weththasinghe et al. (2021); (4) Fisher et al. (2020); (5) Devic et al. (2018); (6) Cummins et al. (2017); (7) Adeoye et al. (2020); (8) Rawski et al. (2020); (9) Biasato et al. (2019); (10) & (11) Zozo et al. (2022). †= defatted BSFL meal; ¶=fullfat BSFL meal; *=most likely defatted BSFL meal. NA=data is not available

The specific type of BSF meal used in these studies was not consistently clarified, making further analysis challenging. Nevertheless, all reports unanimously highlight that glutamine comprises the largest portion among the dispensable amino acids in BSF meal, a finding consistent with both tested BSFL meals in the present study.

The essential amino acids content of both types of BSFL meal in this study exhibits a similar trend to dispensable amino acids, with higher composition in defatted BSFL meal compared to full-fat BSFL meal. However, the values of essential amino acids in this study were lower than those reported in previous studies (Magalhães et al. 2017; Rawski et al. 2020; Zulkifli et al. 2022). The amino acid profile of defatted BSF meal in the present study closely resembles the frass product

from BSF reported by Yildirim-Aksoy et al. (2020), raising questions about the purity of the tested commercial BSFL meal.

Table 23. The indispensable amino acid compositions of several types of BSF meal

| Indispensable amino acids (%) | | | | | | | | | | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Arg | His | Iso | Leu | Lys | Met | Phe | Thr | Try | Val | |
| 1.3 | 1.7 | 2.4 | 3.4 | 3.9 | 1.1 | 2.1 | 1.8 | 0.9 | 2.9 | Adeoye et al. (2020) ¶ |
| 1.0 | 1.5 | 0.6 | 1.2 | 0.8 | 1.5 | 1.2 | 1.1 | 1.3 | 0.5 | Yildirim-Aksoy et al. (2020) ¶¶ |
| 2.5 | 2.8 | 2.4 | 3.6 | 3.6 | 1.1 | 2.1 | 1.9 | 3.1 | 3.1 | Zulkifli et al. (2022) ¶ |
| 2.0 | 1.4 | 1.7 | 2.9 | 2.3 | 0.7 | 1.7 | 1.6 | 0.6 | 2.4 | Spranghers et al. (2017) ¶ |
| 2.3 | 1.5 | 1.9 | 3.2 | 2.7 | 0.6 | 1.6 | 1.7 | 0.5 | 2.6 | Cummins et al. (2017) * |
| 2.0 | 1.2 | 2.7 | 2.9 | 2.7 | 0.7 | 1.7 | 1.7 | NA | 2.6 | Devic et al. (2018) ¶ |
| 3.0 | 1.9 | 2.2 | 4.2 | 3.8 | 1.2 | 2.3 | 2.4 | 0.9 | 3.7 | Fisher et al. (2020) * |
| 2.5 | 3.1 | 3.0 | 1.4 | 2.6 | 0.7 | 0.7 | 2.0 | 3.5 | 2.2 | Biasato et al. (2019) † |
| 2.4 | 0.9 | 2.2 | 3.5 | 3.6 | 2.1 | 1.7 | 2.5 | NA | 3.9 | Lan et al. (2022)¶ |
| 2.6 | 1.6 | 2.2 | 3.6 | 3.7 | 1.2 | 2.3 | 2.2 | NA | 3.7 | Li et al. (2017) † |
| 5.0 | 3.0 | 4.9 | 7.2 | 4.8 | 1.9 | 3.9 | 4.6 | NA | 5.9 | Magalhães et al. (2017) ¶ |
| 5.4 | 3.2 | 4.7 | 7.8 | 6.8 | 2.1 | 7.7 | 4.4 | NA | 6.7 | Rawski et al. (2020) |
| 1.6 | 0.8 | 1.5 | 2.9 | 2.1 | 0.6 | 1.5 | 1.3 | 0.7 | 1.9 | Weththasinghe et al. (2021) |
| 2.2 | 1.2 | 1.9 | 2.9 | 2.1 | 6.5 | 1.7 | 1.7 | NA | 2.7 | Schiavone et al. (2017)† |
| 2.7 | 1.6 | 2.4 | 3.7 | 2.5 | 8.7 | 2.2 | 2.2 | NA | 3.5 | Schiavone et al. (2017) †† |

†= defatted BSFL meal; ¶=fullfat BSFL meal; ¶¶= BSF frass; *=most likely defatted BSFL meal. NA=data is not available

The defatting process is anticipated to result in a higher protein content relative to lipid content in BSF meal. However, experiments by Li et al. (2017) and Biasato et al. (2019) suggest that defatted BSF may have a similar amino acid profile to full-fat BSF meal (Devic et al. 2018; Baco et al. 2022; Zulkifli et al. 2022). This suggests that even after the defatting process, the amino acid composition of BSF meal may remain relatively unchanged. In contrast, Schiavone et al. (2017) reported changes in amino acid content in BSF after the defatting process. Their findings

indicated that highly defatted BSF resulted in higher amino acid content compared to partially defatted BSF, supporting the results of the present study.

Table 24. The dispensable amino acid compositions from different sources of BSF

| Amino acids | References | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) |
| Alanine | 3.9 | 3.1 | 2.5 | 3.7 | 2.4 | 3.3 | 7.7 | 2.4 | 3.6 | 4.4 |
| Aspartate | 4.6 | 5.1 | 3.7 | NA | 4.5 | 5.3 | 9.3 | 3.2 | 3.7 | 4.9 |
| Cysteine | 2.4 | 0.2 | 0.3 | 0.8 | 0.5 | NA | 2.4 | 0.2 | 0.1 | 0.2 |
| Glutamine | 5.6 | 6.1 | 4.2 | 6.4 | 6.8 | 5.6 | 11.6 | 4.2 | 4.9 | 6.4 |
| Glycine | 2.6 | 0.2 | 2.3 | 3.5 | 2.4 | 2.6 | 5.1 | 1.5 | 2.4 | 3 |
| Proline | 2.8 | 2.9 | 2.3 | 3.4 | 3.1 | NA | 5.9 | 1.9 | 3.1 | 3.3 |
| Serine | 2.2 | 2.1 | 1.7 | 2.4 | 2.2 | 2 | 7.1 | 1.2 | 2 | 2.7 |
| Tyrosine | 4.3 | 3.1 | NA | 3.8 | NA | 3.4 | 5.8 | 1.8 | 2.6 | 3.4 |

(1) Adeoye et al. (2020), (2) Zulkifli et al. (2022), (3) Spranghers et al. (2017), (4) Fisher et al. (2020), (5) Lan et al. (2022), (6) Li et al. (2017), (7) Magalhães et al. (2017), (8) Weththasinghe et al. (2021), (9) Schiavone et al. (2017), (10) Schiavone et al. (2017). NA=data is not available

In aquafeeds, ensuring an adequate supply of lipids or fats is crucial to meet the balanced nutritional requirements of the cultured species. Beyond just the lipid content, the composition of fatty acids within it holds significance. BSF meal is recognized as a good lipid source for fish when provided in appropriate amounts (Xu et al. 2020). However, there are limited studies analyzing the fatty acids composition in BSFL meal, primarily due to the substantial variability influenced by factors such as the age of BSF larvae (Ewald et al. 2020), substrate (Ewald et al. 2020; Lan et al. 2022), and processing methods (Hender et al. 2021). Hender et al. (2021) reported that the defatting process of BSFL improves the crude protein content per kilogram of dry matter. Conversely, the composition of fatty acids in partially defatted BSFL meal decreased after the defatting process. This study similarly found that the fatty acids content of full-fat BSFL meal is higher than that of defatted BSFL meal. The reduction in total crude lipid is positively correlated with the decrease in fatty acids

content. Therefore, the choice of defatting process can be tailored based on the desired final nutritional contents. For instance, a partial defatting process may be applied to achieve moderate fat content, while a highly defatting process is suitable for substantial lipid removal from BSFL.

Table 25. The fatty acid compositions of several types of BSF meal

| Fatty acids | References | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (1a) | (1b) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
| C12:0 | 53.8 | 47.3 | 17.8 | 21 | 36.4 | 45.9 | 57.3 | 19.5 | 14.7 | 34.5 |
| C14:0 | 8.9 | 8.6 | 5.2 | 3.7 | 6.8 | 8.7 | 7.3 | 4.5 | 3.1 | 39.9 |
| C16:0 | 20.5 | 14.1 | 20.6 | 5.3 | 15.4 | 12.2 | 9.6 | 21.0 | 19.4 | 16.3 |
| C16:1 n7 | 6.2 | 4.4 | 1.7 | 1.6 | 2.4 | 1.9 | 1.9 | 2.5 | 3.5 | 2.6 |
| C18:0 | 4.6 | 3.1 | 2.9 | 0.8 | 1.4 | 2.5 | 1.4 | 3.9 | 4.1 | 2.1 |
| C18:1 n7 | NA | NA | ND | 6.1 | 0.6 | ND | 2.4 | 24.0 | 21.7 | 0.4 |
| C18:2 n6 | 0.1 | ND | 24.8 | 3.8 | 11 | 14.1 | 11.5 | 19.6 | 28.6 | 9.9 |
| C18:3 n6 | 0.2 | 0.1 | ND | 0.5 | 0 | 1.6 | 0.7 | 1.1 | 1.9 | NA |
| C20:0 | 0.1 | ND | ND | <0.1 | 0.1 | 0.1 | 0 | 0.2 | 0.3 | NA |
| C20:2 | NA | NA | ND | 0.1 | ND | ND | ND | NA | 0.4 | NA |

†= defatted BSFL meal; ¶=fullfat BSFL meal; *=most likely defatted BSFL meal. NA=data is not available; ND=not detected. DF=defatted; FF=Fullfat. (1a) (Hender et al. 2021) (1b) Hender et al. (2021); (2) Zulkifli et al. (2022); (3) Rawski et al. (2020); (4) Rabani et al. (2019); (5) Daszkiewicz et al. (2022); (6) Spranghers et al. (2017); (7) Chen et al. (2021); (8) Lan et al. (2022); (9) Schiavone et al. (2017)

Both types of BSFL meal tested in this study exhibit a rich composition of palmitic acid (C16:0), oleic acid (C18:1), and linoleic acid (C18:2), similar to findings from other studies on BSFL meal (Lan et al. 2022; Zulkifli et al. 2022). Since the substrate used for commercial BSF larvae in this study is unknown, the comparable pattern of fatty acids composition may explain this situation. While Lan et al. (2022) used tofu as a substrate and Zulkifli et al. (2022) utilized agro-industry waste for growing BSF larvae, both studies resulted in similar fatty acids composition as presented above.

Table 26. Mineral composition of BSFL from different sources

| Minerals | | | | | | | References |
|---|---|---|---|---|---|---|---|
| Ca | Na | Mg | Zn | Fe | Mn | Cu | |
| 8.2 | 1.1 | 4.5 | 172 | 154 | 203 | - | Biasato et al. (2019) |
| 16 | 8 | 2 | 22.5 | - | 185.1 | 7.8 | Fisher et al. (2020) |
| 28.7 | 0.7 | 2.7 | 160.0* | 350.0* | 220.0* | 10.0* | Spranghers et al. (2017) |
| 21.4 | 1.3 | 3.9 | 131 | 204 | 232 | 11.2 | Boykin (2019) |
| 18.5 | - | 2.8 | 110.0* | 150.0* | 120.0* | - | Campbell et al. (2020) |
| 26.5 | 5.2 | 3.3 | 303.1 | 300.7 | 140.1 | 13.7 | Zulkifli et al. (2022) |

*The value for Ca (calcium), Na (natrium), & Mg (magnesium) are presented as g/kg. The value of Zn (zinc), Fe (iron), Mn (manganese), and Cu (copper) are presented as mg/kg. (*) values are converted; (-) data is not available.

Minerals are important chemical compounds that are required by animals, including fish for supporting optimal growth. Among several minerals, calcium is known as the most abundant mineral found in animal bodies. In this study, the quantities of calcium, natrium/sodium and magnesium were larger than other minerals and in agreement with previous reports (Spranghers et al. 2017; Biasato et al. 2019; Fisher et al. 2020; Zulkifli et al. 2022). In fact, those mineral compounds are known as major elements. The calcium carbonate in the exoskeleton is responsible for the high calcium constitutes in BSFL. In addition, a small amount of zinc, iron, and manganese was also found in BSFL meal with copper having the least amount. The mineral substances evaluated in commercial defatted BSF in this study were higher than full fat BSF. Thus, the defatting process might improve the purity of BSFL nutrition. A similar finding was also reported by Zozo et al. (2022). However, although the trend of nutritional composition reported is the same with this study, the calcium proportion was not normal, and it was also found that the natrium content in the defatted BSF is lower than in the full fat BSF. It is difficult to draw that conclusion with conflicting available data. For instance, Biasato et al. (2019) reported that the minerals content of partially defatted BSF has a calcium content of less than 10 g/kg, much lower than the calcium of full fat BSF (Spranghers et al. 2017; Fisher et al. 2020; Zulkifli et al. 2022). That anomaly might be associated with the fact that

apart from the defatting process, the origin of the BSF substrate also has a high impact on its nutritional composition.

### 5.5. Summary

The present study indicated that the nutritional value including proximate composition, amino acids, fatty acids, and minerals of commercial full fat and defatted BSFL meal meet the requirement for aquafeeds. In addition, our findings showed that the nutritional value of BSF meal is influenced by the processing type. Generally, the full fat BSFL meal has low protein content and high fat level while the defatted BSFL meal has high protein content and low-fat content. The results can be used as valuable information in improving our knowledge of the exploitation of BSF meal and their suitability for specific aquatic species which considerably progressing such as spiny lobster.

# CHAPTER 6. The effect of defatted black soldier fly (*Hermetia illucens*) meal inclusion on the physiology, hepatopancreas histology and immunology-relatives gene of juvenile lobster (*Panulirus ornatus*)

## 6.1. Introduction

Since the 1980s, dietary insect meal has been recognized as an alternative to fishmeal in livestock food and aquaculture. With the increase in awareness of sustainability related to the use of fishmeal in aquaculture, research on insect meal is also demanding. The potential use of insect-based ingredients in aquadiets has been reviewed (Riddick 2014; Sánchez-Muros et al. 2016; Henry, Gai, et al. 2018; Priyadarshana et al. 2021) and one of the most researched insect species is the black soldier fly (*H. illucens*) (Sánchez-Muros et al. 2016; Priyadarshana et al. 2021; Mohan et al. 2022). The BSF meal has sufficient nutritional content as a substitute for the fish meal as the main protein source in fish and shrimp feed. The use of BSF is used as a total substitute for fishmeal in combination with several protein sources other than fishmeal. Proximate analysis of BSF indicates that amino acids and fatty acids in BSF have comparable nutritional values to fish meal (Mohan et al. 2022). The dietary use of BSF as a protein source is dependent on its life cycle stages as the nutritional profile of BSF varies according to its life stage and the culture medium (Priyadarshana et al. 2021). In general, processed BSF used among fish species' diets are in the forms of maggot meal, sun-dried, oven-dried, frozen, and chopped (Henry, Gasco, et al. 2018) and cultured by using substrates including common organic waste (Shumo et al. 2019) and industrial waste (Ravi et al. 2020).

The utilization of black soldier fly (BSF) meal in finfish diets has been extensively investigated by various researchers (Belghit et al. 2019; Abdel-Tawwab et al. 2020; Hu et al. 2020; Hender et al. 2021; Weththasinghe et al. 2021). However, there are limited nutritional studies exploring BSF meal as a protein source for crustaceans, with only a few instances such as those conducted on shrimp (Cummins et al. 2017; Chen et al. 2021; Richardson et al. 2021). Notably, research on the inclusion of BSF meal in the diet of *L. vannamei* indicated that growth promotion was

ineffective up to 36%, with optimal growth observed at a 7% inclusion rate (Cummins et al. 2017). Similar findings suggested that the ideal substitution level of fishmeal with BSF meal for L. vannamei ranges from 7.5 to 10% (Chen et al. 2021; Richardson et al. 2021). In the case of freshwater lobster, a combination of BSF meal and poultry by-product meal has been identified as beneficial for enhancing growth and immunological gene performance in Australian native freshwater crayfish, *C. cainii* (Foysal et al. 2019). Although ornate spiny lobster (*P. ornatus*) cultivation has been successful in certain countries like Indonesia and Vietnam, the lack of a commercially available formulated diet poses challenges. Despite the nascent stage of formulated diet development for *P. ornatus*, improvements in nutritional studies have been observed, leading to enhanced growth performance under both laboratory and outdoor conditions (Kurnia et al. 2017; Marchese et al. 2018). Nevertheless, there is a notable gap in research regarding the potential use of BSF meal as a protein source for juvenile spiny lobsters. Consequently, the current study aims to assess the viability of incorporating BSF meal into a formulated diet for juvenile spiny lobsters.

## 6.2. Materials and Methods

### Experimental outline

The study employed a research design involving four dietary treatments and four replicates. Juvenile lobsters were raised in 16 rectangular acrylic fish tanks, each with a capacity of 60 liters (60 cm × 29.5 cm × 35 cm), over an eight-week period. Within each tank, 10 juvenile lobsters (initial weight 0.24 ± 0.01 g, initial length 21.77 ± 0.32 mm) were individually placed in 1.5 L plastic baskets with holes (15 cm height; Ø 12.5 cm). To mitigate stress and cannibalism, the baskets were furnished with hides made of polyurethane plastic (4 cm; Ø 1.5 cm) and plastic netting (4 cm × 4 cm). All tanks received filtered water, a continuous air supply, and maintained a natural photoperiod of 12 hours light and 12 hours dark. Throughout the feeding experiment, water quality parameters were monitored using a digital water quality meter (Horiba U-5000, Japan).

Four formulated feeds containing fishmeal replacements at 0%, 25%, 35%, and 50% with a P/E ratio of 26 g CP $MJ^{-1}$ were prepared and assigned as BSF0, BSF25, BSF35, and BSF50 respectively (Table 27). A proximate test was performed to analyse the composition of formulated diets (Table 29) and the raw material of the fishmeal and defatted BSF meal (Table 27). Tested diets were given as moist diets and distributed using stainless steel pincers three times a day until satiation. All uneaten feed was removed after 1 hour of feeding to maintain the water quality. A proximate and amino acid analysis was carried out to determine the composition of the fishmeal and defatted BSF meal as presented in the Table 28 below.

Table 27. Feed ingredients of the formulated diet. Fishmeal replacement using black soldier fly meal in g/500 g ingredients dry weight.

| Ingredients | FM replacement (%) | | | |
|---|---|---|---|---|
| | 0 (BSF0) | 25 (BSF25) | 35 (BSF35) | 50 (BSF50) |
| Fishmeal[1] | 324.00 | 243.00 | 210 | 162 |
| BSF meal[2] | 0 | 81.00 | 113.4 | 162 |
| Astaxanthin | 1.00 | 1.00 | 1.00 | 1.00 |
| Binder | 11.00 | 11.00 | 11.00 | 11.00 |
| Sodium caseinate[3] | 95.50 | 95.50 | 95.50 | 95.50 |
| Wheat | 35.00 | 35.00 | 35.00 | 35.00 |
| Corn starch | 12.50 | 12.50 | 12.50 | 12.50 |
| Cholesterol | 2.00 | 2.00 | 2.00 | 2.00 |
| Fish oil | 6.50 | 6.50 | 6.50 | 6.50 |
| **Total** | **500.00** | **500.00** | **500.0** | **500.0** |

In every 100 g of ingredients, there is the addition of Vitamin C 0.5 g, Soy lecithin 0.5 g, Trace mineral premix 0.5 g and Vitamin premix 1 g. **Vitamin mix**: retinol 180 IU/kg; ascorbic acid, 40 mg/kg; cholecalciferol 40 IU/kg; menadione 2 mg/kg, α-tocopherol 20 mg/kg, choline 200 mg/kg, inositol 200 mg/kg, thiamine/B1 3 mg/kg, riboflavin/B2 4 mg/kg, pyridoxine/B6 3 mg/kg, d-pantothenic acid/B5 10 mg/kg, nicotinic acid 15 mg/kg, biotin 0.1 mg/kg, cyanocobalamin/B12 0.01 mg/kg, folic acid 1 mg/kg. **Mineral mix**: $CoCl_2$ $6H_2O$ 0.5 mg/kg, $CuSO_4$ $5H_2O$ 5 mg/kg, $FeSO_4$ $7H_2O$ 50 mg/kg, KI 4 mg/kg, $CrCl_2$ $6H_2O$ 0.1 mg/kg, $MgSO_4$ $7H_2O$ 150 mg/g, $MnSO_4$

$H_2O$ 25 mg/kg, $NaSeO_3$ 0.1 mg/kg, $ZnSO_4$ $7H_2O$ 100 mg/kg. [1]Fishmeal: local fishmeal with minimum protein of 56%, other ingredients are commonly used ingredients in fish feed manufacturers, sourced from Fish Nutrition Department, IPB University, Bogor – Indonesia. [2]Local defatted BSF meal (Protein 55%, crude lipid 9%, carbohydrate 6% and ash 10%). [3]Sodium caseinate, Friesland Campina-The Netherland

Table 28. Proximate and Amino acids composition of fishmeal and defatted BSF meal (% dry-matter basis).

| *Proximate composition* | FM | DF BSF | % Difference |
|---|---|---|---|
| Crude protein | 56.4 | 55.0 | -2.48% |
| Crude Lipid | 5.4 | 9.0 | 66.67% |
| Ash | 10.40 | 10.90 | 4.81% |
| Moisture | 9.04 | 7.10 | -21.46% |
| *Amino acid* | | | |
| Aspartate | 2.425 | 1.835 | -0.24 |
| Glutamine | 4.432 | 3.120 | -0.30 |
| Serine | 0.806 | 0.712 | -0.12 |
| Glycine | 1.370 | 0.835 | -0.39 |
| Histidine | 0.977 | 0.717 | -0.27 |
| Arginine | 0.916 | 0.692 | -0.24 |
| Threonine | 1.192 | 0.815 | -0.32 |
| Alanine | 0.903 | 0.719 | -0.20 |
| Proline | 1.204 | 0.843 | -0.30 |
| Tyrosine | 0.926 | 0.713 | -0.23 |
| Valine | 0.798 | 0.610 | -0.24 |
| Methionine | 0.687 | 0.522 | -0.24 |
| Cysteine | 0.722 | 0.619 | -0.14 |
| Isoleucine | 1.176 | 0.878 | -0.25 |
| Leucine | 2.243 | 1.297 | -0.42 |
| Phenylalanine | 0.830 | 0.685 | -0.17 |
| Lysine | 1.797 | 1.260 | -0.30 |

% difference was calculated as $(AA_{DBSF} - AA_{FM})/AA_{FM}*100$ (Cummins et al. 2017)

All feed ingredients were mixed and processed by using a food processor (Oxone OX-861N, Indonesia). The finished feed was then stored in a zipped plastic bag until further usage. In this study, the juvenile lobsters were given each type of formulated feed in form of moist diets, four times per day at 15% of wet weight. All uneaten feed was siphoned after 1 hour of feeding to maintain the water quality.

**Proximate analysis of feed and the whole-body of juvenile lobsters**

The proximate composition of the juvenile lobster and the diets were analyzed using the established method in Chapter 3, section 3.5.2. Biochemical analysis

**Intestine and Hepatopancreas Histopathology**

The histopathology of the intestine and hepatopancreas of juvenile lobster were assessed following method in Chapter 3, section 3.5.4. histopathology analysis.

**Gene Expression Analysis**

The histopathology of the intestine and hepatopancreas of juvenile lobster were assessed following method in Chapter 3, section 3.5.5. Immune-relative gene expression analysis.

**Growth and moulting Data**

The physiological data including survival rate and specific growth rate of the juvenile lobster were collected at the beginning and the end of the feeding experiment according to Chapter 3, section 3.5.1. 3.5.1. Evaluation of growth performances.

### 6.2.6. Statistical analysis

The biological data parameters were analysed by using SPSS IBM for Windows (version 25.0) according to Chapter 3, section 3.5.6. Statistical analysis.

## 6.3. Results

In this study, all formulated diets have similar proximate composition, where the differences of value of each nutrition among the diet types were not high (Table 29). The crude protein content ranges from 54.90% in formulated diet BSF35 to 56% in formulated diet BSF0 and BSF50. Crude lipid and ash content of the test diets were 7.01-8.54% and 8.21-8.80%, respectively. The moisture ranged from 11.20% (BSF25; BSF50) to 15.21% (BSF0).

Table 29. Proximate composition of formulated diet (dry-matter basis)

| Composition | Formulated diets | | | |
|---|---|---|---|---|
| | BSF0 | BSF25 | BSF35) | BSF50 |
| Dry matter% | 84.79 | 88.21 | 87.15 | 88.80 |
| Ash% | 8.80 | 8.50 | 8.39 | 8.21 |
| Crude protein% | 56.74 | 55.76 | 54.90 | 56.67 |
| Crude lipid% | 7.36 | 7.01 | 8.20 | 8.54 |
| Moisture (%) | 15.21 | 11.79 | 12.85 | 11.20 |
| Gross energy (g MJ $kg^{-1}$) | 20.73 | 20.64 | 20.61 | 20.56 |

**Amino acids profile**

The amino acid contents in fishmeal were higher compared to the amino acid in the defatted BSF meal (Table 30). The amino acid content was higher in the diet without defatted BSF supplementation compared to supplemented-BSF diets. As the inclusion level of defatted BSF meal in the diet increased, the amino acid content was also increased.

Table 30. Amino acid contents in the experimental diets (% dry weight basis)

| Amino acids | BSF0 | BSF25 | BSF35 | BSF50 |
|---|---|---|---|---|
| Alanine | 0.888 | 0.745 | 0.782 | 0.819 |
| Aspartate | 2.511 | 1.945 | 2.099 | 2.144 |
| Glutamine | 4.712 | 3.566 | 3.787 | 4.313 |
| Serine | 0.810 | 0.762 | 0.740 | 0.755 |
| Glycine | 1.533 | 0.914 | 0.964 | 1.120 |
| Tyrosine | 0.955 | 0.812 | 0.876 | 0.945 |
| Proline | 1.325 | 0.973 | 1.006 | 1.177 |
| Histidine | 1.175 | 0.786 | 0.811 | 0.856 |
| Arginine | 1.024 | 0.722 | 0.792 | 0.981 |
| Threonine | 1.217 | 0.901 | 0.930 | 1.032 |
| Valine | 0.844 | 0.656 | 0.723 | 0.776 |
| Methionine | 0.719 | 0.515 | 0.532 | 0.580 |
| Cysteine | 0.854 | 0.622 | 0.654 | 0.692 |
| Isoleucine | 1.290 | 0.931 | 0.963 | 1.066 |
| Leucine | 2.366 | 1.433 | 1.640 | 1.745 |
| Phenylalanine | 0.953 | 0.677 | 0.732 | 0.780 |
| Lysine | 1.810 | 1.303 | 1.440 | 1.510 |

### 6.3.1. Physiological responses

Physiological evaluation indicated that there were no significant differences in the mean of final weight, specific growth rate, final length, length increment, survival rate and moulting rate of juvenile lobster fed dietary defatted BSF meal (P>0.05). In addition, the number of moults of individual lobsters was also the same (Figure 5). In the present study, optimum responses of lobster juveniles were observed when lobsters received a basic diet with dietary fishmeal as the protein resource. In general, the physiological parameters of juvenile lobsters decreased with the increase of defatted BSF meal inclusion in the tested diets (Table 31).

Table 31. Physiological parameters of juvenile lobster (initial weight 0.24 ± 0.01 g, initial length 21.77± 0.32 mm) fed dietary defatted BSF meal as protein resources. Values are presented in mean ± SE.

| Parameters | BSF0 | BSF25 | BSF 35 | BSF50 |
|---|---|---|---|---|
| Final weight (g) | 0.65 ± 0.06 | 0.64 ± 0.03 | 0.61 ± 0.07 | 0.65 ±0.05 |
| SGR (g% day$^{-1}$) | 1.81 ± 0.13 | 1.77 ± 0.14 | 1.82 ± 0.25 | 1.77 ± 0.03 |
| Final length (mm) | 27.67 ± 0.67 | 28.14 ± 0.59 | 27.78 ± 0.61 | 28.19± 0.76 |
| Length increment (%) | 21.29 ± 1.16 | 22.87 ± 1.59 | 23.08 ± 2.47 | 22.82 ± 0.83 |
| Survival rate (%) | 50.00 ± 0.07 | 50.00 ± 0.07 | 54.17 ± 0.11 | 62.50 ± 0.07 |
| Moulting rate (%) | 62.50 ± 0.12 | 54.17 ± 0.11 | 66.67 ± 0.16 | 58.33 ± 0.04 |

### 6.3.2. Body Composition

The composition of protein, lipid, and protein in the juvenile lobster whole-body were not significantly different (P>0.05). The dietary BSF50 diet resulted in a higher protein and lipid level and the least moisture content compared to other diets. The ash and SOD of juvenile lobster whole-body fed the dietary BSF50 was significantly high (P<0.05) (Table 32).

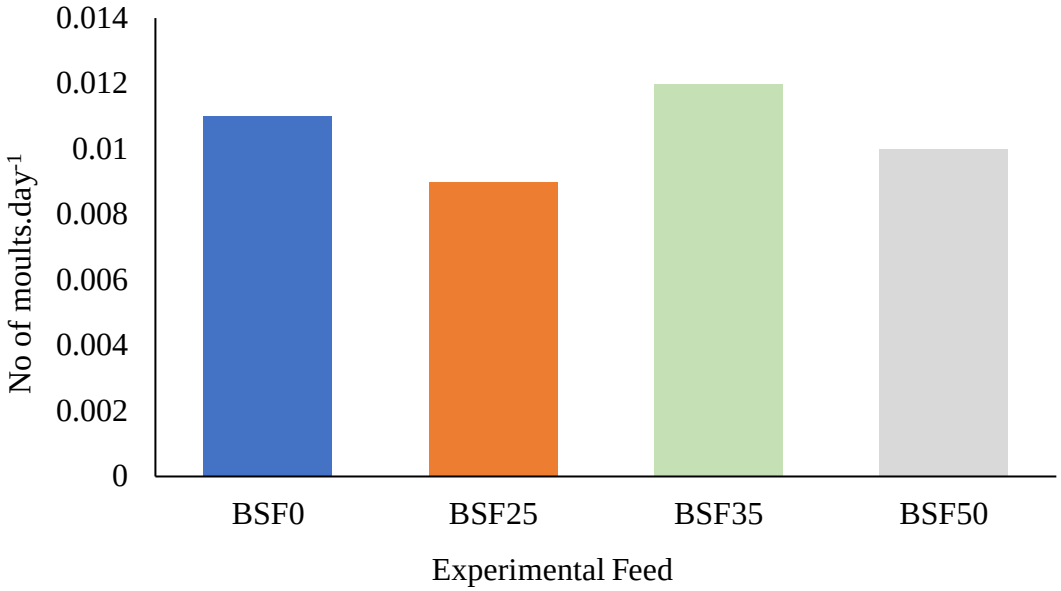



Figure 5. The number of moults of juvenile lobster (initial weight 0.24 ± 0.01 g, initial length 21.77± 0.32 mm). Values are means of N=3 replicates each experimental diet

Table 32. The whole-body wet weight proximate composition of juvenile lobster (initial weight 0.24 ± 0.01 g, initial length 21.77± 0.32 mm) fed defatted BSF meal as protein resources for 56 days.

| Parameters | BSF0 | BSF25 | BSF 35 | BSF50 |
|---|---|---|---|---|
| Protein (%) | 7.90 ± 0.05 | 7.91 ± 0.09 | 7.24 ± 0.04 | 8.67 ± 0.13 |
| Lipid (%) | 0.84 ± 0.03 | 0.93 ± 0.03 | 0.94 ± 0.02 | 0.97 ±0.01 |
| Moisture (%) | 80.33 ± 0.20 | 80.00 ± 0.07 | 81.08 ± 0.55 | 79.75 ± 0.29 |
| Ash (%) | 11.26 ± 0.15$^{a}$ | 12.06 ± 0.11$^{a,b}$ | 13.01 ± 0.11$^{b}$ | 13.59 ± 0.73$^{b}$ |
| SOD | 7.77 ± 0.27$^{a}$ | 10.93 ± 0.21$^{c}$ | 8.97 ± 0.07$^{b}$ | 12.21 ± 0.09$^{d}$ |

Values are presented as means ± SE of N=3 replicates from each treatment. Data in the similar row with different superscripts ($^{a, b}$) indicated a significant difference at $P<0.05$.

### 6.3.3. Relative genes expression

The relative expression levels of cytokine genes in juvenile lobster are shown in Figure 6. In this study, the gene expression of IL-1β and IL-17 in juvenile lobster fed BSF25 and BSF35 were higher than those in juvenile lobster fed BSF0 in terms of fold changes. The IL-17 cells in juvenile lobster given BSF25 and BSF35 showed gene expression five folds that of control (BSF0). In contrast, those cytokine cells decreased as the juvenile lobster fed the dietary BSF50. (Figure 6). The juvenile lobster given dietary BSF50 has a higher fold of ProPO, GPO and ALFHa-1 gene expression compared to other dietary. On the contrary, the ALFHa-2 gene of juvenile lobsters decreases as the increase of the inclusion of BSF levels in the formulated feed

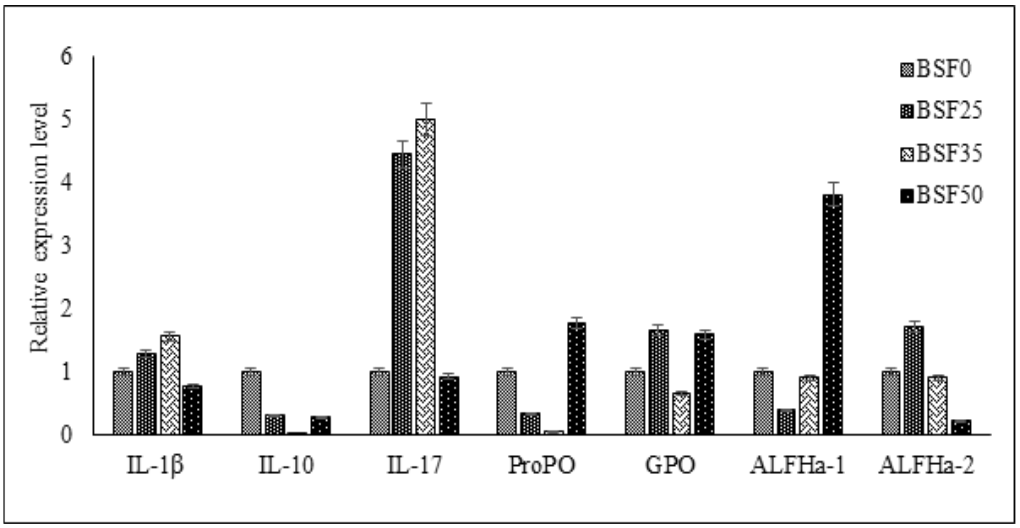



Figure 6. Relative expression level of cytokines gene (IL-1β , IL-10 and IL-17) and immunological genes (ProPO, GPO, ALFHa-1, and ALFHa-2) of juvenile lobster with respect to defatted BSF meal supplementation.

**Hepatopancreas histopathology**

Histology analysis of juvenile lobster fed dietary fishmeal and BSF meal as their protein sources indicated that necrosis on the hepatopancreas tissue was observed on the lobster fed 50% fishmeal replacement (Figure 7). The infiltration of haemocytes and sloughing of the hepatopancreas tissue was found in all treatments. The atrophy of hepatopancreas cells was found in lobster juveniles fed dietary BSF50.

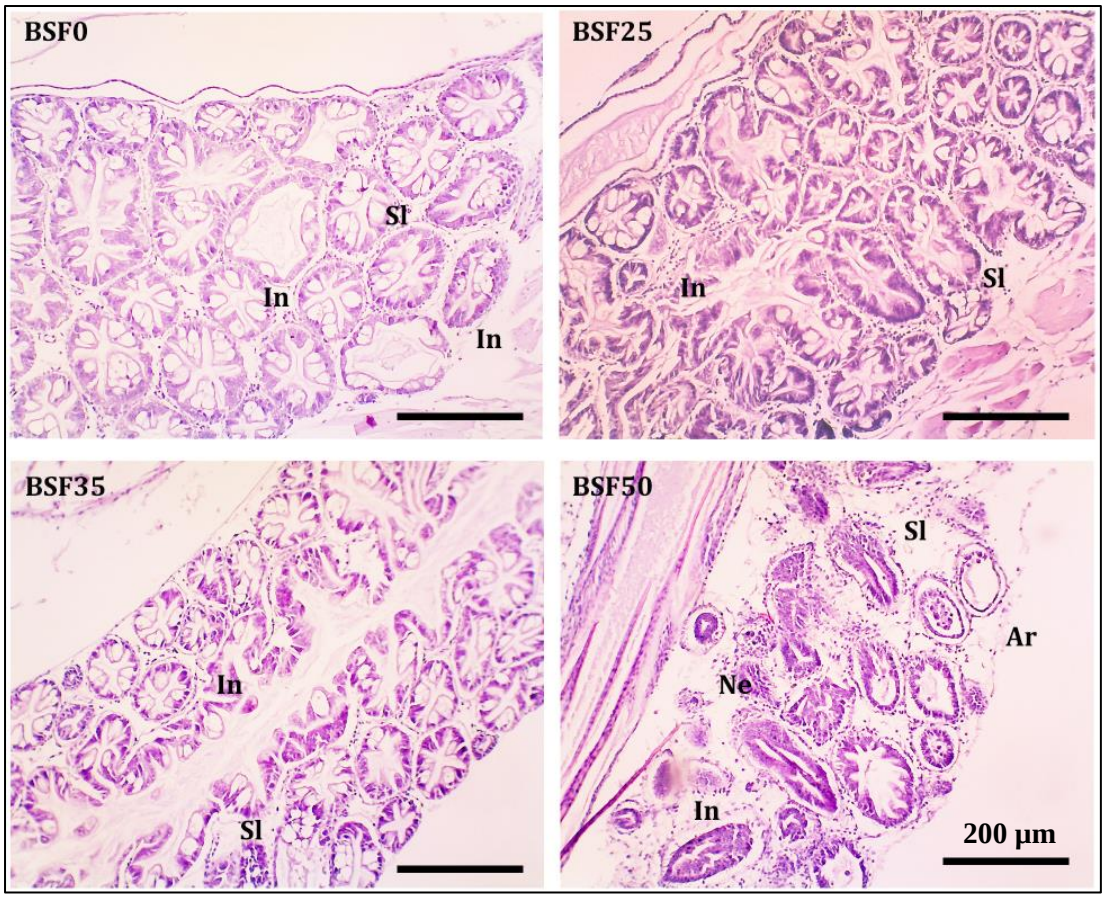



Figure 7. Histopathology of the hepatopancreas of juvenile lobster fed dietary defatted BSF meal inclusion (25%, 35% and 50%) at 100x magnification. Hepatopancreas tissue infiltrations and sloughing occur in all of the treatments. Hepatopancreas of juvenile lobster fed BSF.

## 6.4. Discussion

Insect meal has been widely used as an alternative feed for fish farming. In crustacean culture, the majority of insect meals derived from *T. molitor* and *H. illucens* have been used as protein sources. Those alternative protein sources replace the fishmeal in the diet of pacific white shrimp, *L. vannamei* (Cummins et al. 2017; Choi et al. 2018; Chen et al. 2021; Richardson et al. 2021), and Australian crayfish, *C. cainii* (Foysal et al. 2019) with varying results. In this study, it was found that the substitution of fishmeal with defatted BSF meal of up to 50% did not affect the growth of juvenile lobsters with a specific growth rate between 1.77-1.82 g% day$^{-1}$. It

is difficult to compare the growth rate of the result in this study since this is the first study in the use of defatted BSF meal as a protein sources of the *P. ornatus* juvenile lobsters' diet. However, the growth rate obtained in this study was higher compared to the result of the feeding experiment on *P. homarus* conducted by Haryanti et al. (2017). In that study, the supplementation of probiotics and yeast improved the growth rate of juvenile lobster at 1.20 – 1.33 g% $day^{-1}$. The replacement of fishmeal with defatted BSF meal in this study also did not affect the final total length, final weight, length increment, survival rate and moulting rate of juvenile lobster even at 50% of replacement. The detrimental effects of the use of BSF meal were reported on the white shrimp *L. vannamei* and the maximum amount of 10% of BSF inclusion is believed to be acceptable (Cummins et al. 2017; Richardson et al. 2021). Based on these results, the use of defatted BSF meal as much as 50% replacing fishmeal can be used as an alternative protein source for juvenile lobster without any adverse impact.

The survival rate of lobster juveniles in the present study was not affected by the various levels of supplementation of defatted BSF meal in the diets. The implementation of the individual rearing system in this study eliminated mortality from cannibalism and represented survival only from the feeding factor. In our observation, the death of juvenile lobster was caused by the unsuccess moulting process which can be seen from the incomplete moult of the juvenile lobster body. That event agrees with the findings reported by Irvin and Williams (2009b). With the survival rate ranging from 50.00 to 62.50%, that value is higher than the survival rate of juvenile lobsters reared using the similar rearing system reported by Kropielnicka-Kruk et al. (2019). In that study, the juvenile lobster reared in the separated system with low and high-water exchanges had survival rates of 43 ± 8.49 % and 46 ± 9.71% respectively. Previously, Marchese et al. (2018) also reported that the survival rate of juvenile lobsters in the individual rearing system resulted in a 30-55% higher survival rate than juvenile lobsters in the communal rearing system. According to studies conducted by Irvin and Williams (2009b), the survival of juvenile lobsters in solitary rearing systems has higher survival than those kept in communal rearing systems. In terms of growth, the communal rearing system allows the juvenile lobster to access

the food which can promote their growth. A similar pattern was also reported in adult lobsters as previously reported by Vijayakumaran et al. (2010).

In this study, the amino acids profile of defatted BSF meal was compared to fishmeal and the result indicated that all amino acids component in the defatted BSF meal was lower than the amino acids in the fishmeal. Of the total of 17 amino acids, three amino acids include serine, cysteine, and phenylalanine in the defatted BSF have the closest disparity to these components in the fishmeal compared to the other amino acids components. In addition, as the increase of the inclusion of defatted BSF meal in the experimental diets resulted of the increase the availability of amino acids in the diets which having closer value to the basic diet. Cummins et al. (2017) reported that, with the exception of histidine and tyrosine, the amino acid composition in menhaden fishmeal was higher when compared to that in full-fat BSF larvae meal. The difference between that result compared to this study may be due to the type of BSF since defatted BSF meal was used in this study while they used fully fatted BSF meal. Those BSF materials have influence in the quality of BSF meal in term of amino acids profile and in accordance with Schiavone et al. (2017) whose reported that amino acids in the highly defatted BSF was higher compared to partially defatted BSF. In addition, the substrate of BSF also has significant influence in the nutritional value of BSF (Shumo et al. 2019).

The body composition analysis of juvenile lobster indicated that the ash and SOD content increased as the increase of the defatted BSF meal, whereas protein, lipid and moisture content were the same. The high ash content in the juvenile lobster fed dietary defatted BSF meal did not have any association with the physiological response of juvenile lobster. It has been reported that the level of ash in the diet has a negative correlation with the feed efficiency of the diet (Shearer et al. 1992).

Histopathological analysis on lobsters can be carried out to determine tissue changes due to disease infection (Shields et al. 2012; Zha et al. 2018). Several features of histological conditions such as hepatopancreas necrosis and haemocyte infiltration were reported in the *H. americanus* lobster which has the characteristics of epizootic shell disease. In general, the histopathological and distinct changes of

several internal organs in lobsters have strong association with the presence of bacterial infection and non-infectious diseases (Zha et al. 2018) and can be used as a reference in assessing the general health condition of lobster (Shields et al. 2012). In this study, the results of histopathological analysis of digestive organs and hepatopancreas were performed. However, the staining process of digestive organs failed in obtaining representative images and only histology of hepatopancreas tissue were discussed. Histology of hepatopancreas tissue indicated that sloughing and infiltration occurred in all juvenile lobster samples from each treatment. A clear atrophy and necrosis hepatopancreas cells were observed in juvenile lobster fed with 50 % of defatted BSF meal (Figure 3). The negative impact occurs on hepatopancreas cells may be due to the overuse of defatted BSF meal. That is because the necrotizing and atrophy cells were not found in lobster juvenile fed BSF25 and BSF35 but BSF50. This indicated that defatted BSF meal has limitation in replacing fish meal in the diet of juvenile lobster. The cells atrophy and necrosis in lobster cells are known as the response of the environmental contamination (Shields et al. 2012).

The gene expression level of juvenile lobster indicated different responses with respect to the defatted BSF meal supplementation in the experimental diets. The supplementation of defatted BSF meal at 25 and 35% in the experimental diet increased the IL-1β and IL-17 cytokine cells of juvenile lobster while 50% of defatted BSF supplementation downregulated both cytokine cells. Similar findings were also reported in freshwater lobster conducted by Foysal et al. (2019). In that study, the supplementation of 30% BSF in the diets improved the cytokine cells include IL-7, IL-10, and IL-17, which coincide with the high availability of lactic acid bacteria from the Firmicutes phylum (Foysal et al. 2019). In addition, the abundances of certain firmicutes are responsible for the increase of the availability of some cytokine cells in fish (Miao et al. 2018). The inclusion of defatted BSF meal in the experimental diet in this study may have important roles in providing the firmicutes phylum which affect the increase of cytokine cells of juvenile lobster. However, it still required further investigation to evaluate the findings.

The addition of yeast and combination of probiotics to the diet improved the availability of several immunity genes in juvenile lobster, *P. homarus* (Haryanti et al. 2017). It that report, the essentials nutrition of the yeast and the function of the probiotics have responsibilities in promoting immunological status of juvenile lobster. In the present study, juvenile lobster receiving high inclusion defeated BSF in the diet, having higher prophenoloxidase, glutathione and anti-lipopolysaccharide factor-1 than others. That finding indicate that high dose of defatted BSF meal may improve some of the immunity genes of juvenile lobster. The prophenoloxidase is a part of innate immunity system in crustacean and has been reported play a significant role in the bacterial infection process (Wang et al. 2010).

## 6.5. Summary

The current research indicates that incorporating defatted black soldier fly (BSF) meal, replacing fishmeal up to 50% in formulated feeds for juvenile lobsters, does not impact growth, survival rate, moulting rate, moult interval, or body composition compared to the standard feed. However, it does bring about notable changes in cytokine cells and the expression of certain immune-related genes in juvenile lobsters. The hepatopancreas of lobsters fed with a diet containing 50% defatted BSF meal exhibits severe cell damage. Nevertheless, an increase in the amount of defatted BSF meal enhances the availability of amino acids in the formulated diets and superoxide dismutase in the whole body of juvenile lobsters. Additional research is needed to validate these findings and explore potential supplements that could enhance the acceptance of defatted BSF meal in the formulated diets of juvenile lobsters.

# CHAPTER 7. The effect of supplementation of defatted, full fat black soldier fly and fish protein hydrolysate on the growth, survival, histopathology, and body composition of juvenile lobsters (*Panulirus ornatus*)

## 7.1. Introduction

The ornate spiny lobster (*P. ornatus*) remains a high value fisheries commodity traded throughout the globe. With an annual value of $5 BN, this is the most expensive among the crustacean commodities (FAO 2022). The life cycle of the *P. ornatus* is complex and undergoes a significant metamorphosis phase to becoming a juvenile lobster (Goldstein 2015). This species inhabits the coastal area in trophic regions (Radhakrishnan et al. 2019). Although it has a valuable potential market, the exploitation of *P. ornatus* should be maintained to sustain the lobster fishing industry. The complete hatchery-based larvae production of *P. ornatus* has been reported for research purposes (Kropielnicka-Kruk et al. 2022) and pilot commercial operations (ornatas.com.au). The production of lobster larvae in a hatchery setting is currently limited for traditional lobster farmers and is a substantial obstacle to lobster aquaculture. In contrast, lobster aquaculture by collecting seedstock (puerulus or early juveniles) from the wild has been practiced since the 1990s in the major lobster producing countries, such as Vietnam and Indonesia (Jones 2010; Priyambodo et al. 2015; Shanks et al. 2015). Currently in lobster aquaculture, the primary feed sourced is trash fish, clams or other bivalves (Irvin and Shanks 2015). The current use of natural feeds in lobster aquaculture provides adequate nutrition requirements for lobster growth (Do Huu and Huong 2014). However, this common feed practice is an environmental issue in terms of sustainability. Therefore, the development of formulated feeds for lobsters is essential to the development of a sustainable industry (Nankervis and Jones 2022).

The black soldier fly (*H. illucens*) or BSF has been considered as one of several types of insects suitable for aquafeeds in shrimps (Riddick 2014; Cummins et al. 2017; Foysal et al. 2019). The nutrition of BSF is influenced by the different substrates provided during their rearing (Adeoye et al. 2020). The BSF reared in

organic waste has a higher value of fat compared to the BSF reared in industrial waste. There are two types of BSF used as feed ingredients in aquaculture including defatted and full-fat BSF meal. Full-fat BSF is largely unprocessed BSF and contains all of the products including fats. It has a high fat content, up to 40 g/100 g dry weight (Lu et al. 2022). This high fat inclusion can negatively impact the growth of fish or crustaceans. Meanwhile, defatted BSF undergoes a fat removal process (Zozo et al. 2022). The process of fat removal can be undertaken using mechanical compression or chemical extraction. Indeed, the defatting process influences the composition of the final product of defatted BSF (Hender et al. 2021; Zozo et al. 2022). Both types of BSF have been reported in several fish crustacean feeding experiments with mixed results. The suboptimum growth and immunity response of the animals following dietary inclusion of BSF meal may be associated with the limitations of BSF digestion (Cummins et al. 2017).

Protein hydrolysate has been reported to improve the digestibility of several feed ingredients and provide optimization of growth results (Hernández et al. 2011; Nguyen et al. 2012; Zhou et al. 2016; De et al. 2020). Protein hydrolysate can be used as a sole protein source or as a supplement in feed formulations. Apart from supporting growth in fish, the incorporation of protein hydrolysate also improved the survival rate, growth and immunity of crustaceans (Quinto et al. 2018; Gunathilaka et al. 2022; Pan et al. 2022). However, it has been reported that the use of protein hydrolysate from squid does not influence the growth of *P. vannamei* (Zhou et al. 2016).

The inclusion of defatted BSF meal in a formulated feed for juvenile lobsters without affecting the physiology was reported. However, the optimum growth and survival of the juvenile lobsters in that study were not achieved (Saputra and Fotedar 2023). Since the growth of juvenile lobsters in that study was suboptimal, the incorporation of additional feed supplement may improve the absorption of BSF nutrition. Some studies are reporting the success of the use of FPH in improving the growth and immunology of crustaceans. However, little is known about the effects in lobster feeds. The present study aims to evaluate protein hydrolysate as supplement in

the feeds of juvenile lobsters, where full-fat BSF meal and defatted BSF meals are utilized as protein sources.

## 7.2. Experimental outline

### 7.2.1. Experimental Design

A total of 200 early juvenile lobsters, *P. ornatus* (initial weight 0.19 ± 0.01 g, initial length 20.13 ± 0.12 mm) were purchased from local fisherman in Lombok, Indonesia. The juvenile lobsters were packed in oxygenated plastic packaging with the density of 10 animals in each pack containing 100 mL seawater, 30 ppt. Upon arrival, all the juvenile lobsters were acclimated in a 180 L container box, equipped with net cuts (50 cm × 25 cm), 60 pipe cuts (Ø 100 mm, 4 cm) and an aeration system. After the 3-day acclimation period, 180 early stage juvenile lobsters were stocked into 18 aquaria (10 animals per aquaria) with a tank volume of 60 L, equipped with individual filtration systems with hides made of pipe and net cuts. In each aquarium, a designated feeding area made of polystyrene (16 cm × 11 cm × 0.3 cm). To ensure the feeding area was in place, it was glued to the bottom of the aquaria using silicone sealant.

### 7.2.2. Feeding experiment

The juvenile lobsters were kept for 8 weeks during the of feeding experiment. Experimental feeds containing supplementation of approximately 25% of fish protein hydrolysate (FPH), full-fat BSF (FF) and defatted BSF (DF) were formulated according to Table 33. In this study, the fishmeal ingredients were sourced from bycatch species *Nemipterus virgatus* with crude protein content of 56%. In addition, the value of fatty acids (Table 34) and amino acids (Table 35) of the BSF and full-fat BSF used in this study was sourced from our previous study (Saputra and Lee 2023). Feed T1 contains fishmeal as a protein source. Feed T2 contains 25% FPH substitutes fishmeal, Feed T3 contains 25% FF, Feed T4 contains 25% DF, Feed T5 contains 25% FPH and 25% DF, and Feed T6 contains 25% FPH and 25% FF. The fatty acids and amino acid content in the defatted and full-fat BSF as the protein sources were presented in Table 34 and Table 35.

All ingredients were mixed through an automatic food processor (Kawano 250G, China). After the completion of mixing, a small amount of water was added to each experimental diet type. The dough of ingredients was then processed further using a food mincer (Oxone OX861n, Indonesia) to mix all ingredients. The fine-formulated mixture was then stored in a sealed plastic bag and kept in a dry place until delivered as moist diets. The experimental diets were given three times per day at 15% wet body weight and the excessive diets were removed from the feeding area. The room condition for the experiment was maintained at 29-30ºC, with the light intensity of 12h day:12h dark and the salinity of the water ranged between 29.44 ± 0.73 ppt.

Table 33. Experimental feed ingredients $g.kg^{-1}$ dry weight basis

| Ingredients | Feed | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Fishmeal | 640 | 576 | 522 | 545 | 502 | 508 |
| Corn starch | 30 | 15 | 30 | 25 | 10 | 10 |
| Wheat (10 CP) | 119 | 68 | 122 | 123 | 66 | 60 |
| Astaxanthin | 2 | 2 | 2 | 2 | 2 | 2 |
| Cholesterol | 4 | 4 | 4 | 4 | 4 | 4 |
| Fish oil | 28 | 28 | 3 | 20 | 25 | 5 |
| Soy lecithin | 5 | 5 | 3 | 3 | 3 | 3 |
| Vitamin premix | 10 | 10 | 10 | 10 | 10 | 10 |
| Mineral premix | 5 | 5 | 5 | 5 | 5 | 5 |
| Binder | 32 | 22 | 32 | 22 | 22 | 22 |
| Vitamin C | 5 | 5 | 5 | 5 | 5 | 5 |
| Casein | 120 | 118 | 130 | 102 | 96 | 120 |
| FPH liquid* | 0 | 142 | 0 | 0 | 128 | 128 |
| BSF full-fat* | 0 | 0 | 132 | 0 | 0 | 118 |
| BSF defatted* | 0 | 0 | 0 | 134 | 122 | 0 |
| DM% | 91.47 | 78.66 | 90.88 | 91.86 | 80.23 | 79.46 |
| Ash% | 8.52 | 8.47 | 8.13 | 8.62 | 8.65 | 8.44 |
| GE MJ/kg* | 18.07 | 17.70 | 18.09 | 18.32 | 17.71 | 17.77 |
| CP% | 47.93 | 47.27 | 47.49 | 48.31 | 47.51 | 47.92 |
| Lipid% | 7.07 | 7.21 | 8.86 | 7.80 | 7.39 | 8.85 |
| P/E Ratio | 26.52 | 26.71 | 26.25 | 26.38 | 26.83 | 26.96 |
| Supplementation (%) | 0 | 24.7 | 25.3 | 24.6 | 24.3 | 25.2 |

*) = data were collected from the feed formulation software.

Table 34. The fatty acids composition of full-fat BSF and defatted BSF meal

| Fatty acids | BSF (g/100 g) | |
|---|---|---|
| | Defatted | Full-fat |
| Caprylic acid | ND | ND |
| Capric acid | ND | ND |
| Lauric acid | 1.23 | 7.21 |
| Myristic acid | 7.52 | 10.23 |
| Palmitic acid | 15.23 | 26.45 |
| Stearic acid | 1.85 | 4.12 |
| Arachidic acid | ND | 0.23 |
| Oleic acid | 17.12 | 23.45 |
| Linoleic acid | 26.12 | 34.32 |
| Linolenic acid | 1.05 | 2.12 |
| Polyunsaturated fatty acids | 27.17 | 36.67 |
| Monounsaturated fatty acids | 18.17 | 25.57 |
| Saturated fatty acids | 25.83 | 48.01 |

Table 35. The amino acids of fishmeal, defatted BSF and full-fat BSF meal

| Amino acid | Protein sources (g/100 g) | | |
|---|---|---|---|
| | FM | DF BSF | FF BSF |
| Aspartate | 2.43 | 1.84 | 2.35 |
| Glutamine | 4.43 | 3.12 | 3.21 |
| Serine | 0.81 | 0.71 | 1.65 |
| Glycine | 1.37 | 0.84 | 1.85 |
| Histidine | 0.98 | 0.72 | 0.43 |
| Arginine | 0.92 | 0.70 | 1.23 |
| Threonine | 1.19 | 0.82 | 0.77 |
| Alanine | 0.90 | 0.72 | 1.28 |
| Proline | 1.20 | 0.84 | 1.82 |
| Tyrosine | 0.93 | 0.71 | 0.78 |
| Valine | 0.79 | 0.61 | 1.27 |
| Methionine | 0.69 | 0.52 | 0.61 |
| Cysteine | 0.72 | 0.62 | 0.74 |
| Isoleucine | 1.18 | 0.88 | 1.18 |
| Leucine | 2.24 | 1.30 | 2.13 |
| Phenylalanine | 0.83 | 0.69 | 0.88 |
| Lysine | 1.79 | 1.26 | 2.16 |

Notes: FPH = Propevia®, crude protein 25.6%, crude fat 4.0%, moisture 57.98%, ash 5.6%. Full-fat BSF = crude protein 40%, crude fat 39.7%, carbohydrate 11.4%, ash 10.97%. Defatted BSF = crude protein 55%, crude fat 9%, carbohydrate 6% and ash 10%.

### 7.2.3. Data Collection and Calculation

At the initial feeding experiment, the means of wet body weight, total length, and carapace length were measured as initial data. At the determination of feeding experiment, the growth data were calculated according to Chapter 3, section 3.5.1. 3.5.1. Evaluation of growth performances.

### 7.2.4. Statistical analysis

The effects of fishmeal replacement with BSFL meal combined with fish protein hydrolysate on specific growth rate, total length increment, carapace length increment, moulting rate and moult frequencies of the lobster juvenile were examined using a one-way ANOVA according to Chapter 3, section 3.5.6. Statistical analysis.

## 7.3. Results

The physiological responses of juvenile lobsters fed FF and DF meal, with and without FPH are presented in Table 36. There were no significant differences in the mean survival rate and moulting rate of the juvenile lobsters among the treatments at the end of the feeding experiment. The survival rate of the juvenile lobsters ranged between 50.0 ± 0.07% and 66.7 ± 0.04%, while the moulting rate was between 62.50 ± 0.13% and 79.17 ± 0.04 %. In contrast, the final body weight, total length, specific growth rate and length increment of juvenile lobster-fed dietary defatted BSF with FPH supplementation were significantly high ($P<0.05$).

The complete proximate composition of juvenile lobster is presented in Table 37. The crude protein content of the juvenile lobsters fed was significantly different ($P<0.05$). The highest crude protein content was found in the juvenile lobster fed FF combined with FPH supplementation (T6). The fat content in juvenile lobster provided the FF feed (T3) was significantly higher than all other treatments ($P<0.05$). In contrast, it has the lowest carbohydrate together with T1 and T6.

Table 36. Physiological responses of juvenile lobster. Initial mean weight 0.21 ± 0.01 g, mean total length 20.53 ± 0.12 mm. Data is presented in the mean ± SE from three replicates. Data in the similar column with different superscripts ($^{a,b}$) represents a significant difference at P<0.05.

| Parameter | Feeds | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| SR | 50.0 ± 0.07 | 45.8 ± 0.04 | 54.2 ± 0.15 | 50.0 ±0.07 | 62.5 ± 0.07 | 66.7 ± 0.04 |
| Final BW | 0.43 ± 0.03$^{a}$ | 0.42 ± 0.01$^{a}$ | 0.41± 0.01$^{a}$ | 0.43 ± 0.03$^{a}$ | 0.46 ± 0.02$^{a,b}$ | 0.51 ± 0.02$^{b}$ |
| Final TL | 25.53 ± 0.32$^{a}$ | 26.72 ± 0.23$^{a,b}$ | 27.70 ± 0.95$^{b}$ | 25.51 ± 0.17$^{a}$ | 28.03 ± 0.95$^{b}$ | 28.21 ± 0.48$^{b}$ |
| SGR | 1.29 ± 0.05$^{a,b}$ | 1.29 ± 0.03$^{a,b}$ | 1.24 ± 0.03$^{a}$ | 1.32 ± 0.08$^{a,b}$ | 1.41 ± 0.03$^{a,b}$ | 1.51 ± 0.05$^{b}$ |
| LI | 24.16 ± 0.01$^{a}$ | 32.75 ± 0.01$^{a,b}$ | 35.16 ± 0.04$^{b}$ | 22.33 ± 0.01$^{a}$ | 36.69 ± 0.03$^{b}$ | 38.12 ± 0.01$^{b}$ |
| MR | 70.83 ± 0.11 | 62.50 ± 0.13 | 75.00 ± 0.12 | 83.33 ± 0.04 | 75.00 ± 0.12 | 79.17 ± 0.04 |

SR=survival rate (%); BW=wet body weight (g); TL=total length (mm); SGR=specific growth rate (% g/day); LI= length increment (%); MR=moulting rate (%).

Table 37. Proximate body composition of juvenile lobster whole body (% wet weight). Data in the similar column with different superscripts ($^{a,b}$) represents a significant difference at P<0.05

| Parameter | Feeds | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| Ash | 11.15±0.07$^{a}$ | 11.22 ±0.09$^{a}$ | 15.60 ±0.12$^{d}$ | 11.62 ± 0.09$^{b}$ | 11.22 ± 0.09$^{a}$ | 12.36 ±0.11$^{c}$ |
| Moisture | 77.89±0.22$^{d}$ | 72.4 ±0.21$^{b}$ | 73.02 ±0.13$^{b}$ | 79.08 ± 0.10$^{e}$ | 76.15 ± 0.18$^{c}$ | 69.17 ±0.29$^{a}$ |
| Carbohydrate | 1.24 ±0.21$^{a}$ | 3.02 ±0.06$^{d}$ | 1.24 ±0.01$^{a}$ | 1.82 ± 0.01$^{b}$ | 2.04 ± 0.02$^{c}$ | 1.27 ±0.01$^{a}$ |
| Fat | 0.25 ±0.01$^{b}$ | 0.24 ±0.01$^{a,b}$ | 0.45 ±0.01$^{d}$ | 0.26 ± 0.01$^{b,c}$ | 0.28 ± 0.01$^{c}$ | 0.22 ±0.01$^{a}$ |
| Protein | 9.48 ±0.26$^{b}$ | 13.13 ±0.35$^{c}$ | 9.71 ±0.23$^{b}$ | 7.23 ± 0.20$^{a}$ | 10.33 ±0.26$^{b}$ | 16.99 ±0.39$^{d}$ |

The fatty acid analysis of the tested feed revealed that linolenic acid, Eicosatrienoic and erucic acid were found only in the feed with fishmeal included as a protein source. Meanwhile, the arachidic and Eicosadienoic acid were found in fishmeal and FF feed (Table 38). In addition, the feed with full fat BSF larvae meal has significantly high saturated fat, unsaturated fat, polyunsaturated fat, monounsaturated fat, omega 3 fatty acids, omega 6 fatty acids, and omega 9 fatty acids.

Table 38. Fatty acids composition of tested feeds g.kg$^{-1}$ wet weight (mean ± SD). Data in the similar column with different superscripts ($^{a,b}$) represents a significant difference at P<0.05.

| Fatty acids | Feed | | | | | |
|---|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | T5 | T6 |
| AA | 0.03±0.00$^{a}$ | 0.05±0.00$^{d}$ | 0.13±0.00$^{b}$ | 0.05±0.00$^{b}$ | 0.07±0.00$^{c}$ | 0.05±0.00$^{b}$ |
| Capric Acid | 0.01±0.00 | ND | ND | ND | ND | ND |
| Lauric Acid | 0.01±0.00$^{a}$ | 0.83± 0.01$^{f}$ | 0.17±0.00$^{b}$ | 0.29±0.01$^{c}$ | 0.34±0.00$^{d}$ | 0.60±0.00$^{e}$ |
| Myristic Acid | 0.14±0.00$^{a}$ | 0.37±0.01$^{d}$ | 0.58±0.01$^{e}$ | 0.23±0.00$^{b}$ | 0.24±0.00$^{b}$ | 0.29±0.01$^{c}$ |
| Pentadecanoic Acid | 0.02±0.00$^{a}$ | 0.04±0.00$^{a}$ | 0.12±0.00$^{e}$ | 0.05±0.00$^{c}$ | 0.05±0.00$^{d}$ | 0.05±0.00$^{c}$ |
| Palmitic Acid | 1.01±0.01$^{a}$ | 1.41±0.01$^{b}$ | 3.17±0.00$^{d}$ | 1.46±0.01$^{b}$ | 1.58±0.01$^{c}$ | 1.41±0.03$^{b}$ |
| Palmitoleic Acid | 0.17±0.00$^{a}$ | 0.24±0.01$^{b}$ | 0.57±0.01$^{d}$ | 0.23±0.00$^{b}$ | 0.27±0.00$^{c}$ | 0.22±0.00$^{b}$ |
| Heptadecanoic Acid 17: | 0.04±0.00$^{a}$ | 0.06±0.00$^{b}$ | 0.18±0.00$^{c}$ | 0.06±0.00$^{b}$ | 0.08±0.00$^{c}$ | 0.06±0.00$^{b}$ |
| Heptadecanoic Acid 17: | 0.02±0.00$^{a}$ | 0.03±0.00$^{c}$ | 0.08±0.00$^{d}$ | 0.03±0.00$^{c}$ | 0.03±0.00$^{c}$ | 0.03±0.00$^{b}$ |
| Stearic Acid | 0.36±0.01$^{a}$ | 0.44±0.01$^{b}$ | 1.11±0.02$^{e}$ | 0.51±0.01$^{cd}$ | 0.52±0.01$^{d}$ | 0.47±0.00$^{bc}$ |
| C_Oleic Acid | 2.55±0.03$^{c}$ | 1.81±0.02$^{a}$ | 4.67±0.02$^{d}$ | 1.93±0.02$^{a}$ | 2.43±0.07$^{c}$ | 2.21±0.01$^{b}$ |
| C_Linoleic Acid | 1.75±0.02$^{e}$ | 0.77±0.02$^{a}$ | 2.16±0.01$^{f}$ | 1.01±0.01$^{c}$ | 1.20±0.02$^{d}$ | 0.87±0.02$^{b}$ |
| Linolenic Acid ω6 | 0.01±0.00 | ND | ND | ND | ND | ND |
| Linolenic Acid ω3 | 0.30±0.01$^{c}$ | 0.10±0.00$^{a}$ | 0.38±0.01$^{d}$ | 0.10±0.00$^{a}$ | 0.16±0.00$^{b}$ | 0.10±0.00$^{a}$ |
| Arachidic Acid | 0.03±0.00 | ND | 0.06±0.00 | ND | ND | ND |
| Eicocyanic Acid | 0.12±0.00$^{e}$ | 0.05±0.00$^{c}$ | 0.20±0.00$^{f}$ | 0.04±0.00$^{b}$ | 0.06±0.00$^{d}$ | 0.02±0.00$^{a}$ |
| Eicosadienoic Acid | 0.06±0.00 | ND | 0.09±0.00 | ND | ND | ND |
| Eicosatrienoic Acid ω3 | 0.02±0.00 | ND | ND | ND | ND | ND |
| Eicosatrienoic Acid ω3( | 0.02±0.00 | ND | ND | ND | ND | ND |
| Arachidonic Acid | 0.03±0.00$^{a}$ | 0.05±0.00$^{b}$ | 0.13±0.00$^{d}$ | 0.05±0.00$^{b}$ | 0.07±0.00$^{c}$ | 0.05±0.00$^{b}$ |
| Eicosatpentaenoic Acid | 0.20±0.00$^{e}$ | 0.10±0.00$^{c}$ | 0.48±0.00$^{f}$ | 0.07±0.00$^{a}$ | 0.13±0.00$^{d}$ | 0.08±0.00$^{b}$ |
| Erucic Acid | 0.01±0.00 | ND | ND | ND | ND | ND |
| Docosahexanoic Acid | 0.27±0.00$^{b}$ | 0.25±0.00$^{ab}$ | 1.03±0.01$^{d}$ | 0.23±0.01$^{a}$ | 0.31±0.01$^{c}$ | 0.25±0.01$^{ab}$ |
| DHA | 0.27±0.00$^{b}$ | 0.25±0.00$^{ab}$ | 1.03±0.01$^{d}$ | 0.23±0.01$^{a}$ | 0.31±0.01$^{c}$ | 0.25±0.01$^{ab}$ |
| EPA | 0.20±0.00$^{e}$ | 0.10±0.00$^{c}$ | 0.48±0.00$^{f}$ | 0.07±0.00$^{a}$ | 0.13±0.00$^{d}$ | 0.08±0.00$^{b}$ |
| Unsaturated Fat | 5.53±0.07$^{d}$ | 3.40±0.00$^{a}$ | 9.80±0.02$^{e}$ | 3.69±0.00$^{b}$ | 4.66±0.05$^{c}$ | 3.83±0.02$^{b}$ |
| ∑SFA | 1.60±0.02$^{a}$ | 3.15±0.04$^{d}$ | 5.38±0.01$^{e}$ | 2.59±0.03$^{b}$ | 2.82±0.01$^{c}$ | 2.88±0.04$^{c}$ |
| ∑PUFA | 2.66 ± 0.03$^{d}$ | 1.27±0.01$^{a}$ | 4.28±0.01$^{e}$ | 1.46±0.02$^{b}$ | 1.86±0.03$^{c}$ | 1.35±0.01$^{a}$ |
| ∑MUFA | 2.86 ± 0.04$^{c}$ | 2.13±0.01$^{a}$ | 5.5± 0.02$^{d}$ | 2.23±0.02$^{a}$ | 2.80±0.08$^{c}$ | 2.48±0.01$^{b}$ |
| ∑n-3 FA | 0.79 ± 0.01$^{d}$ | 0.45±0.00$^{b}$ | 1.90±0.00$^{e}$ | 0.40±0.01$^{a}$ | 0.60±0.01$^{c}$ | 0.43±0.01$^{ab}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| ∑n-6 FA | 1.81 ± 0.02 $^{e}$ | 0.82±0.01 $^{a}$ | 2.29±0.01 $^{f}$ | 1.06±0.01 $^{c}$ | 1.27±0.01 $^{d}$ | 0.92±0.02 $^{b}$ |
| ∑n-9 FA | 2.56 ± 0.03 $^{c}$ | 1.81±0.02 $^{a}$ | 4.67±0.02 $^{d}$ | 1.93±0.02 $^{a}$ | 2.43±0.07 $^{c}$ | 2.21±0.01 $^{b}$ |
| Linoleic Acid ω6 | 1.75 ± 0.02 $^{e}$ | 0.77±0.01 $^{a}$ | 2.16±0.01 $^{f}$ | 1.01±0.01 $^{c}$ | 1.20±0.02 $^{d}$ | 0.87±0.02 $^{b}$ |
| Oleic Acid | 2.55 ± 0.03 $^{c}$ | 1.81±0.02 $^{a}$ | 4.67±0.02 $^{d}$ | 1.93±0.02 $^{a}$ | 2.43±0.07 $^{c}$ | 2.21±0.01 $^{b}$ |
| Linolenic Acid | 0.31 ± 0.01 $^{c}$ | 0.10±0.00 $^{a}$ | 0.38±0.01 $^{d}$ | 0.10±0.00 $^{a}$ | 0.16±0.00 $^{b}$ | 0.10±0.00 $^{a}$ |
| Linoleic Acid | 1.75 ± 0.02 $^{e}$ | 0.77±0.01 $^{a}$ | 2.16±0.01 $^{f}$ | 1.01±0.01 $^{c}$ | 1.20±0.02 $^{d}$ | 0.87±0.02 $^{b}$ |

ND=not detected

The fatty acids analysis of the juvenile lobster's whole body indicated that only several fatty acids were found including palmitic, palmitoleic, stearic, oleic, and linoleic acid. Those fatty acid compounds in juvenile lobsters provided the FF feeds were significantly higher than the others ($P<0.05$). In addition, palmitoleic was only found in juvenile lobsters fed dietary non-fish protein hydrolysate supplemented feed (Table 39).

Table 39. Fatty acids composition of juvenile lobster whole body g.kg$^{-1}$ wet weight (mean ± SE). Data in the similar column with different superscripts ($^{a,b}$) represents a significant difference at $P<0.05$.

| **Fatty Acids** | **Feeds** | | | | | |
|---|---|---|---|---|---|---|
| | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** |
| Palmitic Acid | 0.84±0.03$^{b}$ | 0.76± 0.02$^{ab}$ | 1.30 ±0.03$^{d}$ | 0.73±0.02$^{a}$ | 0.99 ±0.03$^{c}$ | 0.77±0.03$^{ab}$ |
| Palmitoleic Acid | 0.20 ± 0.00 | ND | 0.42 ± 0.00 | 0.30± 0.01 | ND | ND |
| Stearic Acid | 0.36± 0.0$^{b}$ | 0.30 ±0.00$^{ab}$ | 0.52± 0.00$^{d}$ | 0.37± 0.01$^{a}$ | 0.36 ±0.01$^{c}$ | 0.32± 0.01$^{ab}$ |
| C Oleic Acid | 0.69±0.02$^{a}$ | 0.91 ± 0.03$^{b}$ | 1.37 ±0.02$^{d}$ | 0.76± 0.02$^{a}$ | 1.02± 0.02$^{c}$ | 0.70± 0.02$^{a}$ |
| C Linoleic Acid | 0.36±0.01$^{a}$ | 0.39 ± 0.01$^{a}$ | 0.84± 0.02$^{b}$ | 0.39±0.01$^{a}$ | 0.38± 0.00$^{a}$ | 0.37± 0.01$^{a}$ |
| Unsaturated Fat | 1.25±0.03$^{b}$ | 1.30 ± 0.03$^{b}$ | 2.63 ±0.05$^{d}$ | 1.45±0.04$^{c}$ | 1.40±0.01$^{bc}$ | 1.06 ± 0.03$^{a}$ |
| ∑SFA | 1.20±0.04$^{b}$ | 1.05 ± 0.03$^{a}$ | 1.82 ±0.03$^{d}$ | 1.10±0.00$^{ab}$ | 1.35± 0.04$^{c}$ | 1.09±0.04$^{ab}$ |
| ∑PUFA | 0.36 ± 0.0$^{a}$ | 0.39 ± 0.04$^{a}$ | 0.84± 0.02$^{b}$ | 0.39± 0.0$^{a}$ | 0.38 ±0.00$^{a}$ | 0.37 ± 0.01$^{a}$ |
| ∑MUFA | 0.89±0.02$^{b}$ | 0.91 ± 0.03$^{b}$ | 1.79± 0.02$^{d}$ | 1.06±0.03$^{c}$ | 1.02 ±0.02$^{c}$ | 0.70 ± 0.02$^{a}$ |
| ∑n-6 FA | 0.36±0.01$^{a}$ | 0.39 ± 0.01$^{a}$ | 0.84± 0.02$^{b}$ | 0.39±0.01$^{a}$ | 0.38 ±0.00$^{a}$ | 0.37± 0.01$^{a}$ |
| ∑n-9 FA | 0.70± 0.02$^{a}$ | 0.91 ± 0.03$^{b}$ | 1.37± 0.02$^{d}$ | 0.76±0.02$^{a}$ | 1.02 ± 0.02$^{c}$ | 0.70 ± 0.02$^{a}$ |
| Linoleic Acid | 0.36± 0.01$^{a}$ | 0.39 ± 0.01$^{a}$ | 0.84± 0.02$^{b}$ | 0.39±0.01$^{a}$ | 0.38 ± 0.00$^{a}$ | 0.37± 0.01$^{a}$ |
| Oleic Acid | 0.70± 0.02$^{a}$ | 0.91 ± 0.03$^{b}$ | 1.37± 0.02$^{d}$ | 0.76±0.02$^{a}$ | 1.02 ± 0.02$^{c}$ | 0.70 ± 0.02$^{a}$ |

ND=not detected.

In this study, the midgut histopathology of juvenile lobsters from T1 and T2 can not be generated due to the sample damage. There were no significant differences in the mean of villus height muscle thickness in the midgut ($P>0.05$). The degeneration of submucosal occurred in the midgut of juvenile lobsters from Figure 8. The hepatopancreas histopathology showed atrophy on the tubules of juvenile lobsters fed with dietary fishmeal, FPH, and DF. The haemocyte infiltration was found in juvenile lobster fed FPH as the protein source. The B and R-cells were active in the hepatopancreas of juvenile lobster fed DF as protein sources. The B-cell was found in hepatopancreas of juvenile lobster fed FF and supplemented with FPH (Figure 9).

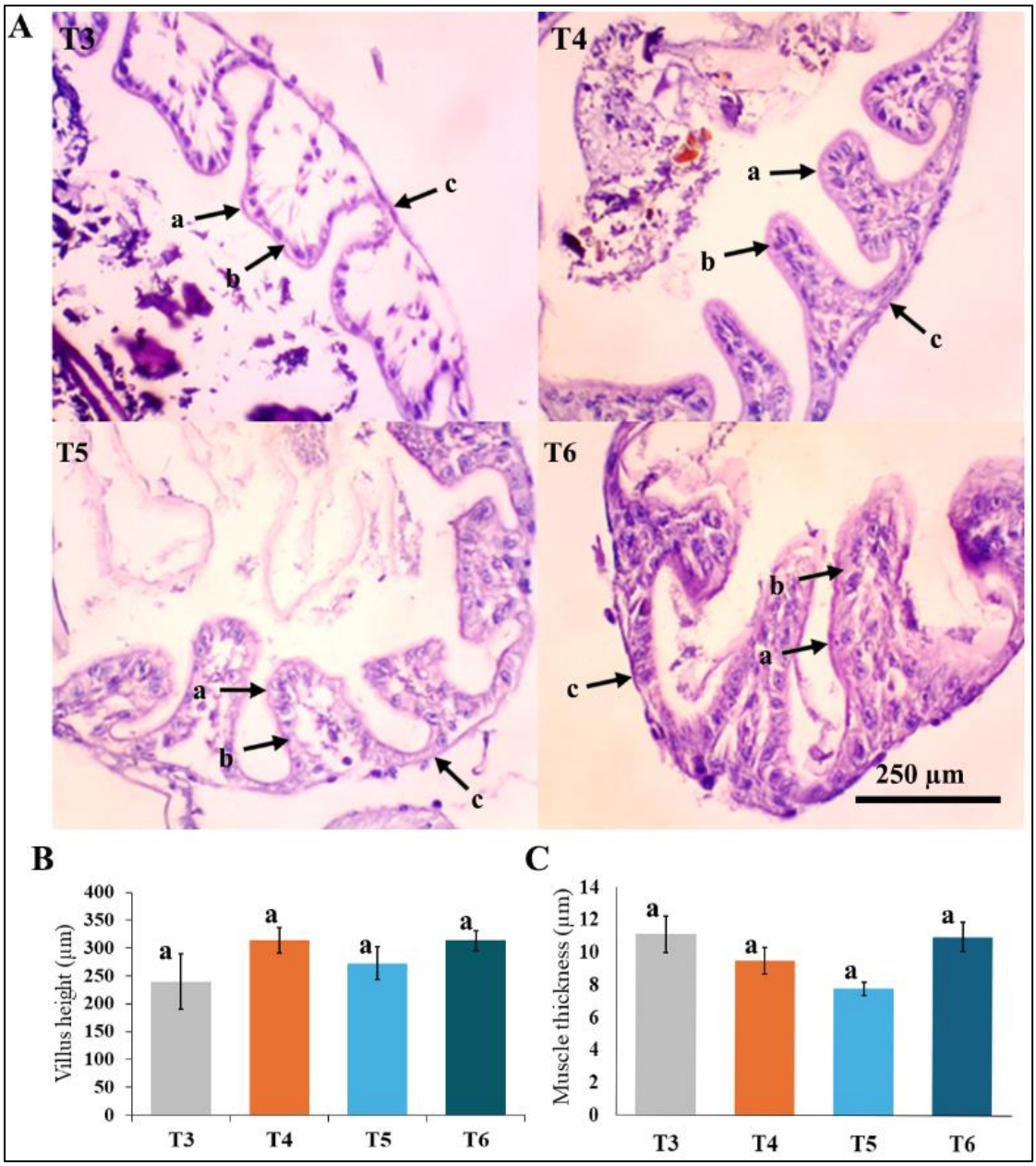



Figure 8. Effect of dietary FPH, defatted BSF, full-fat BSF and combination of them on intestine morphology of juvenile lobster (*P. ornatus*). (A) Intestinal histopathology variation by HE staining ; a, intestinal peritrophic membrane; b, epithelial cells: c, muscle layer. (B) The villus height of the intestinal tissue. (C) The thickness of the muscle layer in the intestinal tissues.

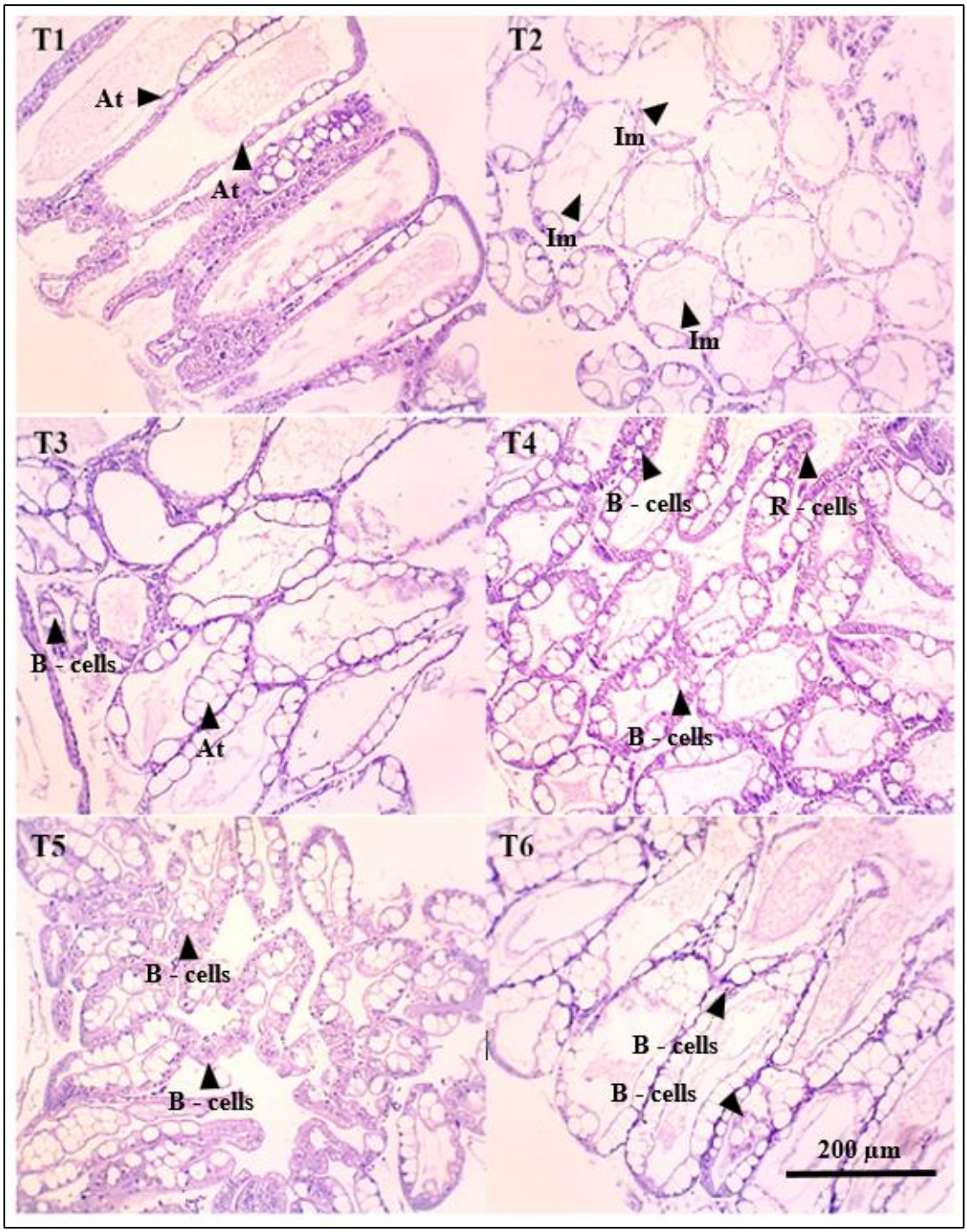



Figure 9. The histopathology of hepatopancreatic tubules in longitudinal and transverse section of juvenile lobsters at 100x magnification (At=atrophy, Im=inflammation)

## 7.4. Discussion

The present study showed that the supplementation of fish protein hydrolysate significantly influenced the most physiological response in of juvenile lobsters. At the supplementation level of 25%, it improves the final total length and specific growth rate. The effectivity of the application of fish protein hydrolysate in crustaceans has been reported (Hernández et al. 2011; Nguyen et al. 2012; Zhou et al. 2016; De et al. 2020; Hlordzi et al. 2022). In this study, the addition of the fish protein hydrolysate to the feeds may improve the absorption of the nutrition in it, which ultimately promoted the growth of juvenile lobsters although the juvenile lobster experienced a suppressed growth rate when compared to other studies (Kropielnicka-Kruk et al. 2019; Kropielnicka-Kruk et al. 2022; Uy et al. 2023). The lower growth rate in this study might be caused by the different source of seed as well as the feed formulation. In this study, the roles of FPH inclusion can be seen in the feeds containing full-fat BSF where the improvement of the growth rate and the final body weight of juvenile lobster occurred. Fish protein hydrolysate have been reported to have several important roles for fish (Latorres et al. 2018; Moreira et al. 2023). Although the inclusion of FPH in the feed for several crustaceans was not significantly affect the growth and survival of the animal (Zhou et al. 2016; Astuti et al. 2023), some other reported the opposite results (Córdova-Murueta et al. 2002; Hernández et al. 2011; De et al. 2020). Zhou et al. (2016) reported that although the protein hydrolysate from squid is a good nutrition source, the inclusion of squid hydrolysate in plant-based diets did not improve the growth of white shrimp. In contrast, recent reports suggest supplementation of 20 ppm protein hydrolysate for *Penaeus vannamei* to reduced the feed by as much as 30% with improvement in the growth, total biomass and survival rates (De et al. 2020).

The survival rates of juvenile lobsters in this study fall within the average survival rates of juvenile lobsters reared under laboratory conditions with an individual housing system (Kropielnicka-Kruk et al. 2019; Kropielnicka-Kruk et al. 2022; Saputra and Fotedar 2023). Those solitary rearing systems can completely prevent the mortality of juvenile lobsters from cannibalism habit and provide

unlimited access of food. However, that limitation also raised concerns about the stress conditions of housed animals which require further investigation. In addition, the lobster social interaction in the communal system may trigger the which stimulate the feeding habit which resulted in a higher growth rate than the individual system (Williams 2009). However, that method allows cannibalism, particularly in the crustacean carnivore species during the moulting period when their health is not at the optimum condition. In this study, to reduce the mortality due to cannibalism and ensure that the mortality was not caused by the cannibalism, some shelters were provided in each aquaria as previously recommended (Slamet et al. 2021).

The results in this study indicated that with the range of 62.50% to 83.33%, the moulting rate of juvenile lobsters in this study was slightly higher than in an earlier study (Saputra and Fotedar 2023). In that study, it was reported that the highest moulting rate was achieved by juvenile lobsters fed with 35% of fishmeal replaced with DF meal. In crustaceans, moulting is a critical process of the growth of animals. At that stage, crustaceans will replace their old exoskeletons with new ones. That process requires immense energy to keep the moulting taking place and determine further development (Lemos and Weissman 2021). In crustaceans, the moulting rate and moulting period of animals have a strong association with the nutrition requirement or other external factors such as temperature (Hewitt and Duncan 2001). In this study, not all juvenile lobsters completed the moulting process, and the moulting rate was the same among the treatments. The incomplete moulting process was observed during the feeding trial, resulting in the mortality of juvenile lobsters. This proved to be the major contributor to the mortality rate in this study. The formulated feed quality might be responsible for that incomplete moulting process since in crustaceans the moulting or ecdysis require a high amount of energy to be completed. In addition, the low moulting rate coincided with the low growth rate of the lobster and required further investigation.

The histopathology of animal organs reflects the general condition of the animal and it has been used widely in assessing the health status of crustaceans (Sreeram and Menon 2005; Shields et al. 2012; Méndez-Martínez et al. 2018;

Ambasankar et al. 2022; Huang et al. 2023) or for other purposes such as assessing the reproduction organ developments (Manan et al. 2022). In this study, noticeable changes in the midgut and hepatopancreas structure of juvenile lobsters were observed. The villus of the midgut is the most important part of the digestive system and increased in terms of size following additional FPH supplementation. In a previous study, the histopathology of hepatopancreas juvenile lobsters was affected by the increase in the inclusion level of DF meal. The hepatopancreas structures of juvenile lobsters that receive dietary DF meal as protein sources above 35% of the replacement have previously demonstrated to sustained damage (Saputra and Fotedar 2023). Another study also reported the change in intestine structure in fish following BSF meal inclusion in the diet (Li et al. 2017). Although the BSF meal is known as a good source of nutrition for animal feed (Abd El-Hack et al. 2020; Priyadarshana et al. 2021) there was a limitation of its usage in the formulated aquafeeds (Li et al. 2017; Chen et al. 2021; Richardson et al. 2021; He et al. 2022) . The mechanism of nutrition absorption of the BSF meal in cultured fish and crustaceans remains unknown. One of the problems identified with the use of BSF meal in aquafeeds is the high ash content. The high content of ash in the BSF larvae meal was reported due to a high mineral composition (Hu et al. 2017). The obvious histological damage of the intestine following the excessive use of BSL meal was reported and that is clear evidence of the limitation of BSF meal utilization (Li et al. 2017; Saputra and Fotedar 2023). The supplementation of chitinase was reported to improve the nutrition of BSF meal in the aquafeed (Agbohessou et al. 2022). In this study, the single use of FPH may not affect the growth and immunology status of juvenile lobsters. However, the availability of FPH in the feed containing the FF and DF meal may improve nutrient absorption due to their biological function as antimicrobial peptides (Halim et al. 2016). In *P. vannamei*, it has been reported that the growth and feed intake of the animals fed FPH were greater than those animals which did not receive FPH. It coincidentally occurs with the fact that the digestive enzyme in the shrimp was also improving as a result of the increased inclusion of FPH in the diet (Hlordzi et al. 2022). In addition, the inclusion of FPH in the feed has been reported, increasing the

abundance and diversity of phytoplankton and zooplankton in the shrimp ponds. Their presence in the pond system is beneficial in maintaining the water quality and also providing additional nutritional sources for penaeid shrimp post larvae which make an optimum favorable condition for shrimps to grow (De et al. 2020).

The BSF meal was not only reported to be as a protein source but also as a lipid source in aquafeeds (Belghit et al. 2019; Ewald et al. 2020; Hender et al. 2021). Research has shown that the Eicosatpentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the fish body were significantly affected by the inclusion of BSL meal in the diets (Belghit et al. 2019). Nonetheless, the inclusion of BSF oil has no influence on the lipid class within the body composition of *Lates calcalifer* (Hender et al. 2021). In this study, although DHA and EPA were found in the tested feed, the proximate composition of the whole body juvenile lobster indicated the absence of them. The ∑SFA in the whole body juvenile lobster was not influenced by the inclusion of BSF meal without FPH supplementation. There was a change in total unsaturated fat, ∑PUFA, ∑MUFA, ∑n-6 FA and ∑n-9 FA. Data from the result also indicated that the replacement of fishmeal using FPH did not influence the general fatty acid profile of the juvenile lobster's whole body. The incorporation of FPH into the feeds containing FF and DF also has equivalent results. The reduction of the amount of fatty acids in the body composition may be due to the typical FPH used in this study, where liquid FPH was used. Similar results were also reported in *Ompok pabda* (Suma et al. 2023). The increase of FPH supplementation in the feeds significantly reduced the amount of total fat in the liver, digestive tract, and muscle of *O. pabda*. Conversely, it increases the amount of total protein as observed in the present study.

## 7.5. Summary

The supplementation of fish protein hydrolysate in the feed containing FF and DF as the main protein sources resulted in the improvement of the growth of juvenile lobsters. The availability of FPH may improve the absorption of nutrients in the feed providing optimum nutrition for the juvenile lobster to grow. However, the histopathological observations on the hepatopancreas and midgut of juvenile lobster indicated that the supplementation of fish protein hydrolysate did not improve the

height of villus and muscle thickness of the intestine. In future feeding experiments on the lobster, additional analysis such as gut microbiome might be extremely useful to support the findings.

# CHAPTER 8. General discussion, conclusions, and recommendations

## 8.1. General Discussion

One of the main factors that determine the sustainability of aquaculture is adequate feed. In carnivorous crustacean species such as spiny lobsters, the use of fresh fish as feed has been widely applied. That practice was believed as the best method for providing optimum nutrition for spiny lobsters in captivity. However, the use of fresh fish as a food source has several constraints including the uncertainty of supply, difficulties of storage, and environmental issues. Therefore, formulated feed for spiny lobsters has been initiated (Smith et al. 2003; Smith et al. 2005). Their findings indicated that formulated feeds for spiny lobsters require high protein levels as well as a high P/E ratio, implying massive use of fishmeal. That because, fishmeal is used as the main source of protein in aquafeed. The dependency of fishmeal usage as a protein source in the diets for future lobster aquaculture may be reduced by introducing alternative protein sources. Until now, nutritional information for spiny lobster for aquaculture purposes is limited and further improvement is needed (Nankervis and Jones 2022). The potential application of fishmeal substitution with insect meal in the aquadiets for fish has been reviewed (Henry et al. 2015; Priyadarshana et al. 2021), and it has been also considered for crustacean aquaculture with some successful results (Riddick 2014). Even though several types of insects have a high crude protein content and are comparable to fishmeal, the use of insect meal as a source of fish protein is not yet optimal and still has several obstacles such as unbalanced nutritional content and unknown levels of acceptability. Therefore, there is a need to develop low-energy formulated feed for spiny lobsters with the use of insect meal and several combinations to support the development of the lobster aquaculture industry.

The P/E ratio has become reference for making formulated crustaceans diets and is believed to be more important than making diets based only on the amount of protein content. In earlier research, the use of formulated diets with high protein

content was introduced, resulting in a high P/E ratio (Smith et al. 2003; Smith et al. 2005). The use of diets with a high P/E ratio has been reported to promote optimum growth for juvenile lobsters. However, it is not practical due to the unsustainability of fish meals. That is because in a high P/E ratio diet, a high amount of protein source, mostly from fish meal is required, thus increasing the dependency of lobster aquaculture on the supply of fish meal. However, several studies at the field level that used diets with a lower P/E ratio showed similar results as in high P/E ratio (Irvin and Shanks 2015). The suitable P/E ratio of the formulated diets has been a critical aspect of aquaculture for several crustaceans (Zhang et al. 2017; Méndez-Martínez et al. 2018; Sudtongkong et al. 2020). In the first experiment of this study (Chapter 4), a feeding trial was carried out on juvenile lobsters to understand the effects of different P/E ratio levels on growth, survival, amino acid composition, and digestive enzymes. The results of the first experiment showed that from the series of P/E ratios given, diets with a higher P/E ratio gave better results, and vice versa, as evidenced by low growth, moulting rate, digestive enzyme, and amino acid content in juvenile lobsters. The current results strengthen the findings in the early study of potential formulated diet for juvenile lobsters, which recommended that a high P/E ratio of formulated diet should be incorporated (Smith et al. 2003; Smith et al. 2005). The poor physiological response of juvenile lobsters to diets with a lower P/E ratio may be due to the lack of energy availability in the diets provided, resulting in suboptimal growth and other physiological factors. Apart from that, the type of protein source used may also contribute to these suboptimal results.

The processing methods of insect meals affect the nutritional content, therefore the second and third experiments were carried out to determine the nutritional content of full fat and defatted BSF meal and their potential substitution of fish meal. Results showed that the protein content of defatted BSF exceeds the amount of protein in the full fat BSF and is closer to the protein content of fishmeal (Hender et al. 2021). Conversely, the full fat BSF has a higher amount of crude lipids reaching 36.20% of total dry matter which is almost triple than the crude lipid in the defatted BSF (Chapter 5). Such high crude lipid content of full fat BSF has been

reported to have an adverse effect on cultured crustaceans such as slow growth, lower survival and higher diets conversion ratio which also occurred in this study where juvenile lobsters were fed with a low P/E diet containing full fat BSF as the protein substitutes (Figure 10. ). In addition, the mineral composition including calcium, natrium, magnesium, zinc, iron, manganese, and copper in the defatted BSF was also higher than in full fat BSF. The three main minerals composition including calcium, natrium and magnesium in both full fat and defatted BSF have comparable substantial nutrition to previous reports (Biasato et al. 2019; Boykin 2019; Campbell et al. 2020; Fisher et al. 2020; Zulkifli et al. 2022). The possible reason for the different values of minerals is based on the substrates for the BSF growth or the process involved (Zozo et al. 2022). In this study, the collected BSF was grown in an organic food leftover substrate where that could potentially increase the composition of calcium.

Results on the second trial (Chapter 5) indicated that the defatted BSF has closer nutritional content to fish meal when compared to full fat BSF. To further evaluate the potential use of defatted BSF in aquafeed, the third (Chapter 6) and fourth experiments (Chapter 7) were conducted to evaluate the optimum inclusion level of the defatted BSF meal in a low P/E ratio diet for juvenile lobsters.

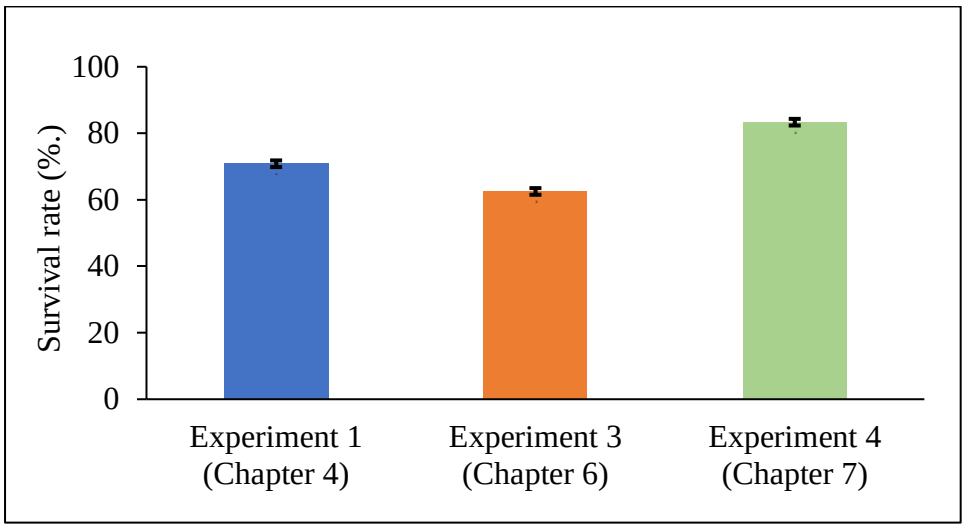



Figure 10. The comparison of the survival rates of juvenile lobsters fed a low P/E ratio supplemented with different protein sources.

The results of the fourth experiment showed that the inclusion of defatted BSF and FPH supplementation in the BSF-based in a low P/E ratio diet improves the survival rate of juvenile lobsters (Figure 10). The survival rate in both studies is high and comparable to the survival rate achieved by juvenile lobsters in previous research (Haryanti et al. 2017; Kurnia et al. 2017; Marchese et al. 2018). In addition, the individual culture system adopted in that study also has an important role in protecting the mortality of spiny lobster juveniles from cannibalism as previously reported (Haryanti et al. 2017; Marchese et al. 2018). Most of the lobster mortality occurred during the process of moulting, where the juvenile lobsters are unable to complete the moulting. The inability to complete the moulting process of the spiny lobster in this study was possibly caused by the lack of energy for the moulting process and in agreement with a previous report (Irvin and Williams 2009a). On the other hand, the inclusion of solely full fat BSF or fish protein hydrolysate as protein sources in a low P/E ratio diet did not improve the survival of juvenile lobsters (Table 40). It seems that the full fat BSF as fishmeal substitutes have unknown drawbacks to the physiological juvenile lobster as also reported by Hu et al. (2020) where nutrition absorption in *Monopterus albus* was not optimal due to the excessive amount of full fat BSF in the diet. Although the survival of juvenile lobsters was suboptimal when full fat BSF was used, it improved the fatty acid composition of the juvenile lobster including total ∑SFA, ∑PUFA and ∑MUFA which is presented in (Table 40).

In terms of growth, results from this study show that the SGR of juvenile lobsters in Chapter 4 was significantly lower than those in Chapters 6 & 7 (Figure 11). Although they have a similar P/E ratio of 26 mg $KJ^{-1}$, there are several factors which could possibly affect that condition. First of all, these differences might be caused by the different sources of protein in the diets. While the experimental diets in Chapter 4 only used fishmeal as the protein source, the diets in Chapter 6 & 7 incorporated of BSF or FPH supplementation in the diets. The inclusion of certain levels of BSF was reported to have important roles in improving the growth immunology condition of crustaceans (Cummins et al. 2017; Foysal et al. 2019; Chen et al. 2021; Richardson et al. 2021). Despite noticeable changes in the digestive tract structure of the animals,

the presence of BSF meal in the diets also improve the availability of beneficial microorganism community and increase the cytokine cells from intestine tissue (Foysal et al. 2019). Secondly, the lobster post pueruli in those experiment were obtained from the different locations which potentially affected the individual variations. The lobster juveniles used in Chapters 6 & 7 were from the same location and differ from the location of collected lobster juveniles in Chapter 4.

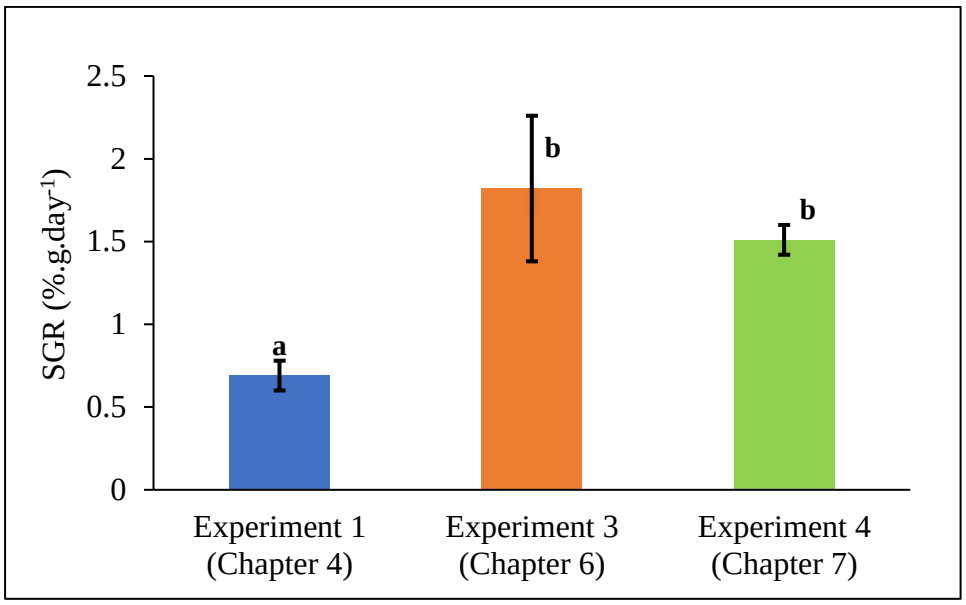



Figure 11. The comparison of the specific growth rate of juvenile lobsters fed a low P/E ratio supplemented with various protein sources. Bars with different superscripts ($^{a,b}$) represent a significant difference at $P<0.05$.

Table 40. The growth, survival and fatty acids composition of juvenile lobsters fed BSF-based diets with and without FPH supplementation. ▲ increased significantly when compared to the control, ▼ decreased significantly when compared to the control and (-) no significant changes compared to the control.

| Parameters | Inclusion level (Chapter 6) | | | Inclusion level (Chapter 7) | | | | |
|---|---|---|---|---|---|---|---|---|
| | DB25 | DB35 | DB50 | 25FP | 25FB | 25DB | $50DB_{25DB+25FP}$ | $50FB_{25FB+25FP}$ |
| WG (%) | - | - | - | - | - | ▼ | ▲ | ▲ |
| SGR (% $g.day^{-1}$) | - | - | - | - | - | ▼ | ▲ | ▲ |
| SR (%) | - | - | - | - | - | - | - | - |
| MI (%) | - | - | - | - | - | - | ▲ | ▲ |
| MR (%) | - | - | - | - | - | - | - | - |
| LI (%) | - | - | - | - | - | - | - | - |
| ∑SFA | NA | NA | NA | ▼ | ▲ | - | ▲ | - |
| ∑MUFA | NA | NA | NA | - | ▲ | ▲ | ▲ | ▼ |
| ∑PUFA | NA | NA | NA | - | ▲ | - | - | - |
| ∑n3-PUFA | NA | NA | NA | - | - | - | - | - |

Note: DB=defatted BSF meal; FP=Fish protein hydrolysate; FB=full fat BSF

In this study, the fishmeal as a protein source in the low P/E ratio diets for juvenile lobsters can be replaced with the defatted BSF meal as much as 50% substitution without affecting the survival rate. However, the histopathology and immune-related gene expression results suggest the optimum inclusion level is at 35% where detrimental effects on the hepatopancreas cells occurred including cell atrophy, sloughing, and cell necrosis (Table 41). In that 35% inclusion level of defatted BSF meal, the gene expression of IL-1β and IL-17 in juveniles was also increased (Chapter 6). Similar results were also reported where the inclusion of 30% BSF in the experimental diets for maron (*C. cainii*) improves the expression of cytokine genes: IL-7, IL-10, and IL-17 (Foysal *et.al.*, 2019). The supplementation of fish protein hydrolysate to the low P/E ratio BSF-based diets has also improved the health of the animal which is reflected by the presence of B-cells in the hepatopancreas and the increase of microvilli size (Table 41). The role of B-cells in crustaceans is for lipid digestion and as recycling cells (Vogt 2019). In addition, the B-cells play an important role in producing antibodies in fish (Kordon et al, 2022). The increase in the number and size of microvilli in the digestive tract has a strong association with the immunological status of crustaceans (Sang and Fotedar 2010; Zhang et al. 2012; Nugroho and Fotedar 2015; Saputra et al. 2019). Hence, the supplementation of fish protein hydrolysate in the low P/E ratio BSF-based diet might improve the nutritional absorption which ultimately provides a balanced nutrition for optimum growth.

Despite differences in survival rates obtained in this study, the specific growth rate of juvenile lobsters was improved in all experiments using the inclusion of BSF meal types or supplemented BSF-based diets with low P/E ratio. The growth of juvenile lobsters fed BSF-based diets with the supplementation of fish protein hydrolysate was also higher (Figure 11). Positive effect of FPH supplementation in a low P/E BSF-based diet was also observed in the moult increment where the supplementation of 25% increased the moult increment significantly as well as increased the availability of ∑SFA and ∑MUFA in the juvenile lobster whole body (Table 40).

Table 41. The histopathological analysis of hepatopancreas and digestive tract of juvenile lobster fed BSF-based diet supplemented with FPH. ▲ increased when compared to the control, (+) the cells or cells characteristic present (-) the cells or cells characteristic absence.

| Observation | Inclusion level (Chapter 6) | | | | Inclusion level (Chapter 7) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | FM | DB25 | DB35 | DB50 | 25FP | 25FB | 25DB | $50DB_{25DB+25FP}$ | $50FB_{25FB+25FP}$ |
| Cell atrophy | - | - | - | + | - | + | - | - | - |
| Hemocyte infiltration | + | + | + | + | + | - | - | - | - |
| Cell Sloughing | + | + | + | + | - | - | - | - | - |
| Cell necrosis | - | - | - | + | - | - | - | - | - |
| B-cells | - | - | - | - | - | - | + | + | + |
| R-cells | - | - | - | - | - | - | + | - | - |
| Microvilli length | NA | NA | NA | NA | - | - | ▲ | ▲ | ▲ |

Note: FM=fishmeal; DB=defatted BSF meal; FP=Fish protein hydrolysate; FB=full fat BSF

The use of fish protein hydrolysate as supplementation in aquadiets has been reported to have a positive effect on the growth of crustaceans (Hernández et al. 2011; Nguyen et al. 2012; Zhou et al. 2016; De et al. 2020) and some unsignificant results were also reported (Astuti et al. 2023). In this study, the use of liquid FPH solely as the main protein source replacing fishmeal did not affect any physiology and immunology of juvenile lobsters. However, when it was used as a supplement to the diets containing full fat and defatted BSF, it resulted in significant growth and immune response improvement of juvenile lobsters (Table 41). Hernández et al. (2011) reported the supplementation of FPH which originated from tuna by-products improved the quality and digestibility of formulated diet for *L. vannamei* containing terrestrial protein. On the other hand, the supplementation of fish protein hydrolysate and shrimp hydrolysate at 3% did not give any enhancement to the growth of the scalloped spiny lobster, *P. homarus* (Astuti et al. 2023).

In this study, under a low P/E ratio, BSF-based diets provide a similar survival rate. However, full fat BSF provides a significantly low growth rate, and full-fat BSF supplemented with FPH diets provides a significantly high growth rate. In diets containing full-fat BSF, the lipid source in the diets mostly comes from BSF meal and fish oil only contributes 0.3% of dry weight basis. The lipid in full fat BSF is known to have a different composition value from the lipid of defatted BSF which may result in suboptimal growth of juvenile lobsters. Based on the proximate composition, the fatty acids in full fat BSF have much higher levels of saturated fatty acids (SFA) than the defatted BSF, where SFA is known as an unhealthy lipid. This result is strengthened by the findings in the result of Chapter 7, where the diets and juvenile lobsters' whole body where both samples have significantly higher SFA content. That nutritional composition may be responsible for the suboptimal growth of juvenile lobsters (Chapter 7). In contrast, the addition of liquid FPH to diets containing full fat BSF reduces the amount of SFA in the diets which has an impact on the juvenile lobster's whole body as well as the improvement on the growth of juvenile lobsters. Since FPH has been reported to have multi-biochemical functions such as antioxidant (Chalamaiah et al. 2012; Latorres et al. 2018) and antimicrobial peptides (Baco et al.

2022) and it is believed that the inclusion of FPH in the formulated diets improves nutrition absorption and promotes the growth of cultured fish or crustaceans.

Our findings revealed that even at a low P/E ratio, the formulated feed can provide sufficient nutrition for post-puerulii spiny lobster to grow, achieving the expected survival rate and growth with minimum inclusion of high-protein fishmeal. This low P/E ratio formulated feed allow the use of affordable fishmeal sources for lobster feed formulation, potentially reducing the cost production of the feed. In addition, the potential use of BSF meal as protein source in the formulated feed can reduce the dependency of fishmeal. However, although the BSF meal are comparable to fishmeal in term of nutritional quality, the future applications of BSF as a protein source for feed formulations for lobster aquaculture is challenging due to the fact that the BSF production is still limited. Since the production of BSF is simple and cheap, it becomes an opportunity in developing those nutritious ingredients for aquaculture purposes, particularly in lobster feed development.

## 8.2. Conclusions

Based on the findings of this study, it can be concluded that:

1. The dietary low P/E ratio of the diets did not improve the survival of juvenile lobsters significantly.
2. The inclusion of BSF meal in the low dietary P/E ratio increased the specific growth rate of juvenile lobsters.
3. The juvenile lobster cannot tolerate the inclusion of a defatted BSF meal of more than 35%. This can be seen from the damage of the hepatopancreas cells from histopathological results. Also, that inclusion increases the expression of several inflammatory cytokine cells.
4. The supplementation of 25% FPH in the defatted and full fat BSF in the low protein to energy ratio diet improves the survival rate, the growth rate, length increment and weight gain of juvenile lobsters. In addition, the FPH supplementation improved the digestive tract (villus height and muscle thickness) and hepatopancreas cell health.

## 8.3. Limitation

1. The juvenile lobster used in the present study is wild caught, making the initial size of the puerulii varied.
2. The histopathology of the digestive tract and hepatopancreas analysis was not performed in all diet experiments due to the insufficient number of surviving animals.
3. In the present study, the juvenile lobster was kept in an individual compartment system that could influence the growth.
4. The present study used commercial BSF meal types and a self-cultured BSF meal may provide more comprehensive results because we exactly know the substrate of growth and the nutritional condition of the harvested BSF.

## 8.4. Recommendations

1. Since the collected juvenile lobster is wild-caught, high variations in their size and age are inevitable. However, it can be reduced by involving a large number of puerulii, measuring and minimising the standard error of size and weight and a period of acclimation.
2. Further study should consider the use of a communal rearing system to evaluate the growth and survival of juvenile lobsters.
3. The digestibility studies of BSF meal types are needed to fully understand the reasons for the different effects of defatted BSF when compared to full fat BSF.
4. In the future feeding study, the assessment of the biochemical mechanism involved in the metabolism of the BSF diet can be as a useful tool to give an understanding of the positive influence on the growth and immunological responses and the negative histological manifestations with BSF protein replacement of 50% and beyond.
5. Further research on FPH to understand its molecular basis for the positive influence on the growth and immunological responses of the lobsters need to be taken.

## References

Abd El-Hack ME, Shafi ME, Alghamdi WY, Abdelnour SA, Shehata AM, Noreldin AE, Ashour EA, Swelum AA, Al-Sagan AA, Alkhateeb M, Taha AE, Abdel-Moneim A-ME, Tufarelli V, & Ragni M. (2020). Black Soldier Fly (*Hermetia illucens*) Meal as a Promising Feed Ingredient for Poultry: A Comprehensive Review. *Agriculture*, *10*(8). https://doi.org/10.3390/agriculture10080339

Abdel-Tawwab M, Khalil RH, Metwally AA, Shakweer MS, Khallaf MA, & Abdel-Latif HMR. (2020). Effects of black soldier fly (*Hermetia illucens* L.) larvae meal on growth performance, organs-somatic indices, body composition, and hemato-biochemical variables of European sea bass, *Dicentrarchus labrax*. *Aquaculture*, *522*, 735136. https://doi.org/10.1016/j.aquaculture.2020.735136

Adeoye AA, Akegbejo-Samsons Y, Fawole FJ, & Davies SJ. (2020). Preliminary assessment of black soldier fly (*Hermetia illucens*) larval meal in the diet of African catfish (*Clarias gariepinus*): Impact on growth, body index, and hematological parameters. *J. World Aquacult. Soc.*, *51*(4), 1024-1033. https://doi.org/10.1111/jwas.12691

Agbohessou PS, Mandiki SNM, Mbondo Biyong SR, Cornet V, Nguyen TM, Lambert J, Jauniaux T, Lalèyè PA, & Kestemont P. (2022). Intestinal histopathology and immune responses following Escherichia coli lipopolysaccharide challenge in Nile tilapia fed enriched black soldier fly larval (BSF) meal supplemented with chitinase. *Fish Shellfish Immunol.*, *128*, 620-633. https://doi.org/10.1016/j.fsi.2022.08.050

Ai Q, Mai K, Li H, Zhang C, Zhang L, Duan Q, Tan B, Xu W, Ma H, Zhang W, & Liufu Z. (2004). Effects of dietary protein to energy ratios on growth and body composition of juvenile Japanese seabass, Lateolabrax japonicus. *Aquaculture*, *230*(1), 507-516. https://doi.org/10.1016/j.aquaculture.2003.09.040

Akiyama DM, Dominy WG, & Association AS. (1989). *Penaeid Shrimp Nutrition for the Commercial Feed Industry*. American Soybean Association. https://books.google.co.id/books?id=aljdQgAACAAJ

Al-Souti A, Gallardo W, Claereboudt M, & Mahgoub O. (2019). Attractability and palatability of formulated diets incorporated with chicken feather and algal meals for juvenile gilthead seabream, *Sparus aurata*. *Aquaculture Reports*, *14*, 100199. https://doi.org/10.1016/j.aqrep.2019.100199

Ambasankar K, Dayal JS, Vasagam KPK, Sivaramakrishnan T, Sandeep KP, Panigrahi A, Raja RA, Burri L, & Vijayan KK. (2022). Growth, fatty acid composition, immune-related gene expression, histology and haematology indices of *Penaeus vannamei* fed graded levels of Antarctic krill meal at two different fishmeal concentrations. *Aquaculture*, *553*, 738069. https://doi.org/10.1016/j.aquaculture.2022.738069

Anh TL, & Jones C. (2015). Status report of Vietnam lobster growout. In CM Jones (Ed.), *Proceedings of the International Lobster Aquaculture Symposium* (Vol. 145, pp. 285). ACIAR

Anizah MR, Saad CR, Kamarudin MS, & Rahim AA. (2017). Effect of dietary protein and protein energy ratio on the growth performance of lemon fin barb hybrid (*Hypsibarbus wetmorei* × *Puntius gonionotus*) larvae. *Iran. J. Fish. Sci.*, *16*(2), 711-721.

AOAC. (2005a). AOAC Official Methods 990.03. Protein (Crude) in Animal Feed. Combustion method. *Association of Official Analytical Chemist*. (Washington DC)

AOAC. (2005b). Official Methods of Analysis. *Association of Official Analytical Chemist*. (Washignton DC)

Arredondo-Figueroa JL, Ponce-Palafox JT, Shirai-Matsumoto K, Pérez-Zavaleta Á, Barriga-Sosa IDLÁ, & Ruiz Luna A. (2013). Effects of including shrimp protein hydrolysate in practical diets on the growth and survival of redclaw crayfish hatchlings *Cherax quadricarinatus* (Von Martens, 1868). *Aquacult. Res.*, *44*(6), 966-973. https://doi.org/10.1111/j.1365-2109.2012.03102.x

Arumugama A, Dineshkumar R, Rasheeq AA, Gowrishankar MP, Murugana S, & Sampathkumar P. (2020). Growth performance of spiny lobster, *Panulirus homarus* (Linnaeus, 1758). *Indian Journal of Geo Marine Sciences*, *49*(5), 812-819.

Astuti N, Sudewi S, Slamet B, Fahrudin F, & Giri N. (2024). The impact of fresh and artificial diet on growth and survival rate of spiny lobster, Panulirus homarus reared in floating net cage. *BIO Web of Conferences*, *87*. 10.1051/bioconf/20248703022

Astuti NWW, Sudewi, Giri NA, Fahrudin, Asih YN, & Slamet B. (2023). Fish and shrimp hydrolysate as additives in lobster feed formulation: effect on growth, survival, feed consumption of spiny lobster (Panulirus homarus). *IOP Conference Series: Earth and Environmental Science*, *1137*(1), 012027. https://doi.org/10.1088/1755-1315/1137/1/012027

Babu SV, Shareef MM, Shetty AP, & Shetty KT. (2002). HPLC method for amino acids profile in biological fluids and inborn metabolic disorders of aminoacidopathies. *Indian J. Clin. Biochem.*, *17*(2), 7-26. 10.1007/bf02867967

Baco N, Oslan SNH, Shapawi R, Mohhtar RAM, Noordin WNM, & Huda N. (2022). Antibacterial activity of functional bioactive peptides derived from fish protein hydrolysate. *IOP Conference Series: Earth and Environmental Science*, *967*(1), 012019. https;//doi.org/10.1088/1755-1315/967/1/012019

Barragan-Fonseca KB, Dicke M, & Loon JJAv. (2017). Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed - a review. *Journal of Insects as Food and Feed*, *3*(2), 105-120. https://doi.org/10.3920/JIFF2016.0055

Belghit I, Liland NS, Gjesdal P, Biancarosa I, Menchetti E, Li Y, Waagbø R, Krogdahl Å, & Lock E-J. (2019). Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*). *Aquaculture*, *503*, 609-619. https://doi.org/10.1016/j.aquaculture.2018.12.032

Bell TA, & Lightner DV. (1988). *A handbook of normal penaeid shrimp histology*. World Aquaculture Society.

BeMiller JN. (2017). Carbohydrate Analysis. In SS Nielsen (Ed.), *Food Analysis Laboratory Manual* (pp. 147-175). Springer International Publishing. https://doi.org/https://doi.org/10.1007/978-3-319-44127-6_11

Bessa L, Pieterse E, Marais J, & Hoffman L. (2020). Why for feed and not for human consumption? The black soldier fly larvae. *Comprehensive Reviews in Food Science and Food Safety*, *19*, 2747-2763. https://doi.org/10.1111/1541-4337.12609

Bessa LW, Pieterse E, Marais J, Dhanani K, & Hoffman LC. (2021). Food Safety of Consuming Black Soldier Fly (*Hermetia illucens*) Larvae: Microbial, Heavy Metal and Cross-Reactive Allergen Risks. *Foods*, *10*(8), 1-20. https://doi.org/10.3390/foods10081934

Biasato I, Renna M, Gai F, Dabbou S, Meneguz M, Perona G, Martinez S, Lajusticia ACB, Bergagna S, Sardi L, Capucchio MT, Bressan E, Dama A, Schiavone A, & Gasco L. (2019). Partially defatted black soldier fly larva meal inclusion in piglet diets: effects on the growth performance, nutrient digestibility, blood profile, gut morphology and histological features. *Journal of Animal Science and Biotechnology*, *10*(1), 1-11. https://doi.org/10.1186/s40104-019-0325-x

Bortolini S, Macavei LI, Saadoun JH, Foca G, Ulrici A, Bernini F, Malferrari D, Setti L, Ronga D, & Maistrello L. (2020). *Hermetia illucens* (L.) larvae as chicken manure management tool for circular economy. *Journal of Cleaner Production*, *262*, 121289. https://doi.org/10.1016/j.jclepro.2020.121289

Boykin KL. (2019). *Assessing the Nutritional Value of Black Soldier Fly Larvae (Hermetia illucens) Used for Reptile Foods* (Publication Number 4927) Louisiana State University ]. https://repository.lsu.edu/gradschool_theses/4927

Campbell M, Ortuño J, Stratakos AC, Linton M, Corcionivoschi N, Elliott T, Koidis A, & Theodoridou K. (2020). Impact of Thermal and High-Pressure Treatments on the Microbiological Quality and In Vitro Digestibility of Black Soldier Fly (*Hermetia illucens*) Larvae. *Animals*, *10*(4), 1-10. https://doi.org/10.3390/ani10040682

Chalamaiah M, Dinesh kumar B, Hemalatha R, & Jyothirmayi T. (2012). Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chem.*, *135*(4), 3020-3038. https://doi.org/10.1016/j.foodchem.2012.06.100

Chen Y, Chi S, Zhang S, Dong X, Yang Q, Liu H, Tan B, & Xie S. (2021). Evaluation of the Dietary Black Soldier Fly Larvae Meal (*Hermetia illucens*) on Growth Performance, Intestinal Health, and Disease Resistance to *Vibrio parahaemolyticus* of the Pacific White Shrimp (*Litopenaeus vannamei*) [Original Research]. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.706463

Choi IH, Joo Min K, Nam Jung K, Jeong Dae K, Chul Park, Jong Hwan P, & Tae ho C. (2018). Replacing fish meal by mealworm (*Tenebrio molitor*) on the growth performance and immunologic responses of white shrimp (*Litopenaeus vannamei*). *Acta Scientiarum Animal Sciences*, *40*. https://doi.org/10.4025/actascianimsci.v40i1.39077

Chuntapa B, Piyatiratitivorakul S, Nitithamyong C, Viyakarn V, & Menasveta P. (1999). Optimal lipid:carbohydrate and protein:energy ratios in semi-purified diets for juvenile black tiger shrimp *Penaeus monodon* Fabricius. *Aquacult. Res.*, *30*(11-12), 825-830. https://doi.org/10.1046/j.1365-2109.1999.00404.x

Córdova-Murueta, Julio Humberto, García-Carreño, & Fernando Luis. (2002). Nutritive value of squid and hydrolyzed protein supplement in shrimp feed. *Aquaculture*, *210*(1), 371-384. https://doi.org/10.1016/S0044-8486(02)00011-X

Cox SL, & Davis M. (2009). An evaluation of potential diets for the culture of postpueruli spiny lobsters *Panulirus argus* (Palinuridae). *Aquacult. Nutr.*, *15*(2), 152-159. https://doi.org/10.1111/j.1365-2095.2008.00578.x

Cui Y, Ma Q, Limbu SM, Du Z, Zhang N, Li E, & Chen L. (2017). Effects of dietary protein to energy ratios on growth, body composition and digestive enzyme activities in Chinese mitten-handed crab, *Eriocheir sinensis*. *Aquacult. Res.*, *48*(5), 2243-2252. https://doi.org/10.1111/are.13061

Cummins VC, Rawles SD, Thompson KR, Velasquez A, Kobayashi Y, Hager J, & Webster CD. (2017). Evaluation of black soldier fly (*Hermetia illucens*) larvae meal as partial or total replacement of marine fish meal in practical diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture*, *473*, 337-344. https://doi.org/10.1016/j.aquaculture.2017.02.022

Cuzon G, & Guillaume J. (1997). Energy and protein : energy ratio. In L. R. D'Abramo, D. E. Conklin, & DM Akiyama (Eds.), *Crustacean nutrition, advances in world aquaculture* (Vol. Volume 6, pp. 51-70). World Aquaculture Society.

D'Abramo LR, Bordner CE, Conklin DE, & Baum NA. (1984). Sterol requirement of juvenile lobsters, Homarus sp [Article]. *Aquaculture*, *42*(1), 13-25. 10.1016/0044-8486(84)90309-0

Daszkiewicz T, Murawska D, Kubiak D, & Han J. (2022). Chemical Composition and Fatty Acid Profile of the Pectoralis major Muscle in Broiler Chickens Fed Diets with Full-Fat Black Soldier Fly (*Hermetia illucens*) Larvae Meal. *Animals*, *12*(4), 1-13. https://doi.org/10.3390/ani12040464

De D, Sandeep KP, Kumar S, Raja RA, Mahalakshmi P, Sivaramakrishnan T, Ambasankar K, & Vijayan KK. (2020). Effect of fish waste hydrolysate on growth, survival, health of Penaeus vannamei and plankton diversity in culture systems. *Aquaculture*, *524*, 735240. https://doi.org/10.1016/j.aquaculture.2020.735240

Devic E, Leschen W, Murray F, & Little DC. (2018). Growth performance, feed utilization and body composition of advanced nursing Nile tilapia (*Oreochromis niloticus*) fed diets containing Black Soldier Fly (*Hermetia illucens*) larvae meal. *Aquacult. Nutr.*, *24*(1), 416-423. https://doi.org/10.1111/anu.12573

Do Huu H, & Huong L. (2014). *Effects of pellet shape and size on production of spiny lobster (Panulirus ornatus)* Spiny lobster aquaculture development in Indonesia, Vietnam and Australia. Proceedings of the International Lobster Aquaculture Symposium held in Lombok,

Indonesia, 22–25 April 2014. ACIAR Proceedings No. 145. Australian Centre for International Agricultural Research: Canberra. 285 pp,

Espinosa-Magaña A, Lozano-Álvarez E, & Briones-Fourzan P. (2017). Resistance to starvation of first-stage juveniles of the Caribbean spiny lobster. *PeerJ*, *5*, e2852. https://doi.org/10.7717/peerj.2852

Ewald N, Vidakovic A, Langeland M, Kiessling A, Sampels S, & Lalander C. (2020). Fatty acid composition of black soldier fly larvae (*Hermetia illucens*) – Possibilities and limitations for modification through diet. *Waste Manage.*, *102*, 40-47. https://doi.org/10.1016/j.wasman.2019.10.014

FAO. (2017). *The world lobster market* (GLOBEFISH Res. Programme, Issue.

FAO. (2020). *The State of World Fisheries and Aquaculture 2020* (Sustainability in action, Issue. ftp://ftp.fao.org/docrep/fao/011/i0250e/i0250e.pdf

FAO. (2022). *Fishery and Aquaculture Statistics* [FAO Fisheries and Aquaculture Division (Online) Updated 2022.]. www.fao.org/fishery/statistics/software/fishstatj/en

Fisher HJ, Collins SA, Hanson C, Mason B, Colombo SM, & Anderson DM. (2020). Black soldier fly larvae meal as a protein source in low fish meal diets for Atlantic salmon (*Salmo salar*). *Aquaculture*, *521*, 734978. https://doi.org/10.1016/j.aquaculture.2020.734978

Foysal J, Fotedar R, Tay CY, & Gupta S. (2019). Dietary supplementation of black soldier fly (*Hermetica illucens*) meal modulates gut microbiota, innate immune response and health status of marron (*Cherax cainii*, Austin 2002) fed poultry-by-product and fishmeal based diets. *PeerJ*, *7*, e6891. https://doi.org/10.7717/peerj.6891

Francis DS, Salmon ML, Kenway MJ, & Hall MR. (2014). Palinurid lobster aquaculture: nutritional progress and considerations for successful larval rearing. *Reviews in Aquaculture*, *6*(3), 180-203. 10.1111/raq.12040

Franco A, Scieuzo C, Salvia R, Petrone A, Tafi E, Moretta A, Schmitt E, & Falabella P. (2021). Lipids from *Hermetia illucens*, an Innovative and Sustainable Source. *Sustainability*, *13*, 1-23. https://doi.org/10.3390/su131810198

Giri N, Irvin S, Haryanti H, Sudewi S, Selamet B, Jones C, & Mujiono M. (2023). Growth Response of Lobster *Panulirus Homarus* Reared in Tanks and in Floating Net Cages Fed with Compound and Fresh Feed. *Sains Malaysiana*, *52*, 2747-2759. https://doi.org/10.17576/jsm-2023-5210-02

Glencross B, Smith M, Curnow J, Smith D, & Williams K. (2001). The dietary protein and lipid requirements of post-puerulus western rock lobster, *Panulirus cygnus*. *Aquaculture*, *199*(1–2), 119-129. http://dx.doi.org/10.1016/S0044-8486(01)00520-8

Goda AMAS. (2008). Effect of dietary protein and lipid levels and protein–energy ratio on growth indices, feed utilization and body composition of freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879) post larvae. *Aquacult. Res.*, *39*(8), 891-901. https://doi.org/10.1111/j.1365-2109.2008.01947.x

Goldstein JS. (2015). *Tropical lobster aquaculture: Bridging the gaps and forging new frontiers* Spiny lobster aquaculture development in Indonesia, Vietnam and Australia. Proceedings of the International

Lobster Aquaculture Symposium held in Lombok, Indonesia, 22–25 April 2014. ACIAR Proceedings No. 145. Australian Centre for International Agricultural Research: Canberra. 285 pp, Canberra.

Goncalves R, Lund I, Gesto M, & Skov PV. (2020). The effect of dietary protein, lipid, and carbohydrate levels on the performance, metabolic rate and nitrogen retention in juvenile European lobster (*Homarus gammarus*, L.). *Aquaculture*, *525*, 735334. https://doi.org/10.1016/j.aquaculture.2020.735334

Gunathilaka BE, Kim S, Herault M, Fournier V, & Lee K-J. (2022). Marine protein hydrolysates as a substitute of squid-liver powder in diets for Pacific white shrimp (*Litopenaeus vannamei*). *Aquacult. Res.*, *53*(11), 4059-4068. https://doi.org/10.1111/are.15907

Guzman, Gaxiola, Rosa, & Torre B. (2001). The effect of dietary protein and total energy content on digestive enzyme activities, growth and survival of *Litopenaeus setiferus* (Linnaeus 1767) postlarvae. *Aquacult. Nutr.*, *7*(2), 113-122. https://doi.org/10.1046/j.1365-2095.2001.00161.x

Haetami K, Junianto, Iskandar, Rita Rostika, & Abun. (2017). Durability and Water Stability of Pellet Fish Supplementation Results pairing Coconut Oils and Hazlenut Oil. *International Journal of Environment, Agriculture and Biotechnology (IJEAB) 2*(3). https://doi.org/10.22161/ijeab/2.3.40

Halim NRA, Yusof HM, & Sarbon NM. (2016). Functional and bioactive properties of fish protein hydolysates and peptides: A comprehensive review. *Trends Food Sci. Technol.*, *51*, 24-33. https://doi.org/10.1016/j.tifs.2016.02.007

Hamidoghli A, Yun H, Shahkar E, Won S, Hong J, & Bai SC. (2018). Optimum dietary protein-to-energy ratio for juvenile whiteleg shrimp, *Litopenaeus vannamei*, reared in a biofloc system. *Aquacult. Res.*, *49*(5), 1875-1886. https://doi.org/10.1111/are.13643

Haryanti, Sembiring Sbm, Sudewi, Widiastuti D, Giri NAG, & Sugama K. (2017). Immunity responses of lobster juvenile, *Panulirus homarus* wtih the use of probiotic in the moist diet. *Jurnal Riset Akuakultur*, *12*(1), 85-97.

He Y, Liu X, Zhang N, Wang S, Wang A, Zuo R, & Jiang Y. (2022). Replacement of Commercial Feed with Fresh Black Soldier Fly (*Hermetia illucens*) Larvae in Pacific White Shrimp (*Litopenaeus vannamei*). *Aquacult. Nutr.*, *2022*, 9130400. https://doi.org/10.1155/2022/9130400

Hender A, Siddik MAB, Howieson J, & Fotedar R. (2021). Black Soldier Fly, *Hermetia illucens* as an Alternative to Fishmeal Protein and Fish Oil: Impact on Growth, Immune Response, Mucosal Barrier Status, and Flesh Quality of Juvenile Barramundi, *Lates calcarifer* (Bloch, 1790). *Biology*, *10*(6), 1-17. https://doi.org/10.3390/biology10060505

Henry M, Gasco L, Piccolo G, & Fountoulaki E. (2015). Review on the use of insects in the diet of farmed fish: Past and future [Review]. *Anim. Feed Sci. Technol.*, *203*, 1-22. https://doi.org/10.1016/j.anifeedsci.2015.03.001

Henry MA, Gai F, Enes P, Perez-Jimenez A, & Gasco L. (2018). Effect of partial dietary replacement of fishmeal by yellow mealworm (*Tenebrio molitor*) larvae meal on the innate immune response and intestinal antioxidant enzymes

of rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunol.*, *83*, 308-313. https://doi.org/10.1016/j.fsi.2018.09.040

Henry MA, Gasco L, Chatzifotis S, & Piccolo G. (2018). Does dietary insect meal affect the fish immune system? The case of mealworm, *Tenebrio molitor* on European sea bass, *Dicentrarchus labrax*. *Dev. Comp. Immunol.*, *81*, 204-209. https://doi.org/10.1016/j.dci.2017.12.002

Hernández C, Olvera-Novoa MA, Smith DM, Hardy RW, & Gonzalez-Rodriguez B. (2011). Enhancement of shrimp Litopenaeus vannamei diets based on terrestrial protein sources via the inclusion of tuna by-product protein hydrolysates. *Aquaculture*, *317*(1), 117-123. https://doi.org/10.1016/j.aquaculture.2011.03.041

Herrera-Salvatierra N, Pascual-Jiménez C, Huchin-Mian JP, Lozano-Álvarez E, Montero-Muñoz J, Briones-Fourzán P, & Rodríguez-Canul R. (2019). Nutritional and immunological evaluation of juvenile spiny lobsters Panulirus argus (Latreille, 1804) (Decapoda: Achelata: Palinuridae) naturally infected with the PaV1 virus. *J. Crust. Biol.*, *39*(2), 162-171. https://doi.org/10.1093/jcbiol/ruy108

Hewitt DR, & Duncan PF. (2001). Effect of high water temperature on the survival, moulting and food consumption of Penaeus (Marsupenaeus) japonicus (Bate, 1888). *Aquacult. Res.*, *32*(4), 305-313. https://doi.org/10.1046/j.1365-2109.2001.00560.x

Hlordzi V, Wang J, Kuebutornye FKA, Yang X, Tan B, Li T, Cui Z, Lv S, Lao T, & Chi S. (2022). Hydrolysed fish protein powder is better at the growth performance, hepatopancreas and intestinal development of Pacific white shrimp (Litopenaeus vannamei). *Aquaculture Reports*, *23*, 101025. https://doi.org/10.1016/j.aqrep.2022.101025

Holthuis LB. (1991). FAO species catalogue, vol. 13: Marine Lobsters of the World. An Annotated and Illustrated Catalogue of Species of Interest to Fisheries Known to Date. *FAO Fish. Synop.*, *125*, 1-292.

Hu J, Li N, Zhao H, He F, Sun Y, Huang Y, & Wang G. (2017). Effects of Substitution of Fish Meal with Black Soldier Fly (*Hermetia illucens*) Larvae Meal, in Yellow Catfish (*Pelteobagrus fulvidraco*) Diets. *Israeli Journal of Aquaculture - Bamidgeh*, *69*. https://doi.org/10.46989/001c.21056

Hu Y, Huang Y, Tang T, Zhong L, Chu W, Dai Z, Chen K, & Hu Y. (2020). Effect of partial black soldier fly (*Hermetia illucens* L.) larvae meal replacement of fish meal in practical diets on the growth, digestive enzyme and related gene expression for rice field eel (*Monopterus albus*). *Aquaculture Reports*, *17*, 100345. https://doi.org/10.1016/j.aqrep.2020.100345

Hu Y, Tan B, Mai K, Ai Q, Zheng S, & Cheng K. (2008). Growth and body composition of juvenile white shrimp, *Litopenaeus vannamei*, fed different ratios of dietary protein to energy. *Aquacult. Nutr.*, *14*(6), 499-506. https://doi.org/10.1111/j.1365-2095.2007.00555.x

Huang P, Cao L, Du J, Gao J, Zhang Y, Sun Y, Li Q, Nie Z, & Xu G. (2023). Effects of Prometryn Exposure on Hepatopancreas Oxidative Stress and Intestinal

Flora in *Eriocheir sinensis* (Crustacea: Decapoda). *Antioxidants*, *12*(8). https://doi.org/10.3390/antiox12081548

Hung LV, Nam DLH, & Dung TV. (2015). Effect of temperature and pH on the activity of digestive enzymes of ornate spiny lobster (*Panulirus ornatus*) and scalloped spiny lobster (*Panulirus homarus*). *Tap Chi Khoa Hoc-Cong NgheThuy San; Review Of Science-Technology Of Fisheries*(1), 23-28

Huo Y-W, Jin M, Zhou P-P, Li M, Mai K-S, & Zhou Q-C. (2014). Effects of dietary protein and lipid levels on growth, feed utilization and body composition of juvenile swimming crab, *Portunus trituberculatus*. *Aquaculture*, *434*, 151-158. https://doi.org/10.1016/j.aquaculture.2014.08.011

Huu HD, & Jones CM. (2014). Effects of dietary mannan oligosaccharide supplementation on juvenile spiny lobster Panulirus homarus (Palinuridae). *Aquaculture*, *432*, 258-264. https://doi.org/10.1016/j.aquaculture.2014.05.013

Iaconisi V, Bonelli A, Pupino R, Gai F, & Parisi G. (2018). Mealworm as dietary protein source for rainbow trout: Body and fillet quality traits. *Aquaculture*, *484*, 197-204. https://doi.org/10.1016/j.aquaculture.2017.11.034

Ido A, Hashizume A, Ohta T, Takahashi T, Miura C, & Miura T. (2019). Replacement of Fish Meal by Defatted Yellow Mealworm (*Tenebrio molitor*) Larvae in Diet Improves Growth Performance and Disease Resistance in Red Seabream (*Pargus major*). *Animals : an open access journal from MDPI*, *9*(3), 100. https://doi.org/10.3390/ani9030100

Ighwela KA, Ahmad AB, & Abol-Munaf aAB. (2013). Water Stability and Nutrient Leaching of Different Levels of Maltose Formulated Fish Pellets. *Global Veterinaria*, *10*, 638-642. https://doi.org/10.5829/idosi.gv.2013.10.6.7278

Irvin S, & Shanks WE. (2015). Tropical spiny lobster feed development: 2009–2013. In CM Jones (Ed.), *Proceedings of the International Lobster Aquaculture Symposium* (Vol. 145, pp. 285). ACIAR

Irvin SJ, & Williams KC. (2006). Nutrition, Feeds and Feeding of Spiny lobsters. *Aqua Feeds : Formulation & Beyond*, *3*(1).

Irvin SJ, & Williams KC. (2007). Apparent digestibility of selected marine and terrestrial feed ingredients for tropical spiny lobster *Panulirus ornatus*. *Aquaculture*, *269*(1–4), 456-463. http://dx.doi.org/10.1016/j.aquaculture.2007.05.013

Irvin SJ, & Williams KC. (2009a). Comparison of the growth and survival of *Panulirus ornatus* seed lobsters held in individual or communal cages. In KC Williams (Ed.), *Spiny lobster aquaculture in the Asia-Pacific region* (Vol. 132, pp. 89-95). Australian Centre for International Agricultural Research. https://www.aciar.gov.au/sites/default/files/legacy/node/11536/ACIAR_PR132%28online%29.pdf

Irvin SJ, & Williams KC. (2009b). *Panulirus ornatus* lobster feed development: from trash fish to formulated feeds. In KC Williams (Ed.), *Spiny lobster aquaculture in the Asia-Pacific region* (Vol. 132, pp. 162). Australian Centre for International Agricultural Research.

Ismail BP. (2017). Ash Content Determination. In SS Nielsen (Ed.), *Food Analysis Laboratory Manual* (pp. 117-119). Springer International Publishing. https://doi.org/10.1007/978-3-319-44127-6_11

Janssen RH, Vincken JP, van den Broek LA, Fogliano V, & Lakemond CM. (2017). Nitrogen-to-Protein Conversion Factors for Three Edible Insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *J. Agric. Food Chem.*, *65*(11), 2275-2278. https://doi.org/10.1021/acs.jafc.7b00471

Jeyakumar VL, N.Ramanathan, & Thommai F. (2004). Influence of natural, animal feeds and artificial pellet feeds on the growth of Spiny Lobster, *Panulirus homarus*. Proceeding of Ocean Life food & Medicine Expo,

Johnston DJ. (2003). Ontogenetic changes in digestive enzyme activity of the spiny lobster, *Jasus edwardsii* (Decapoda; Palinuridae). *Mar. Biol.*, *143*(6), 1071-1082. http://dx.doi.org/10.1007/s00227-003-1154-0

Johnston DJ, Aj R, Cw T, & Jeffs A. (2004). Digestive enzyme profiles of spiny lobster *Jasus edwardsii* phyllosoma larvae. *Marine Ecology-progress Series 275*, 219-230. https://doi.org/10.3354/meps275219

Johnston DJ, Calvert KA, Crear BJ, & Carter CG. (2003). Dietary carbohydrate/lipid ratios and nutritional condition in juvenile southern rock lobster, *Jasus edwardsii*. *Aquaculture*, *220*(1-4), 667-682. https://doi.org/10.1016/s0044-8486(02)00562-8

Johnston DJ, Ritar AJ, & Thomas CW. (2004). Digestive enzyme profiles reveal digestive capacity and potential energy sources in fed and starved spiny lobster (*Jasus edwardsii)* phyllosoma larvae. *Comp. Biochem. Physiol. B: Biochem. Mol. Biol.*, *138*(2), 137-144. http://dx.doi.org/10.1016/j.cbpc.2004.02.013

Johnston M, Johnston D, & Jones C. (2008). Evaluation of partial replacement of live and fresh feeds with a formulated diet and the influence of weaning *Panulirus ornatus* phyllosomata onto a formulated diet during early ontogeny. *Aquacult. Int.*, *16*, 33-47. https://doi.org/10.1007/s10499-007-9121-2

Johnston MD. (2006). *Feeding and Digestion in the Phyllosoma Larvae of Ornate Spiny Lobster, Panulirus ornatus (Fabricius) and the Implications for their culture* The University of Western Australia]. Perth.

Jones C. (2009a). Temperature and Salinity Tolerances of the Tropical Spiny Lobster, *Panulirus ornatus*. *J. World Aquacult. Soc.*, *40*. https://doi.org/10.1111/j.1749-7345.2009.00294.x

Jones C. (2010). Tropical rock lobster aquaculture development in Vietnam, Indonesia and Australia. *J. Mar. Biol. Ass. India*, *52*, 304-315. https://researchonline.jcu.edu.au/41323/1/Jones%20%236164.pdf

Jones C. (2020). *Research for Development of Lobster Growout Technology in Indonesia* (FR2020-015). ACIAR.

Jones C, & Shanks S. (2009). Requirements for the aquaculture of Panulirus ornatus in Australia. In KC Williams (Ed.), *Spiny lobster aquaculture in the Asia-Pacific region* (Vol. 132, pp. 162). Australian Centre for International Agricultural Research.

Jones CM. (2009b). Advances in the culture of lobsters. In G Burnell & G Allan (Eds.), *Woodhead Publishing Series in Food Science, Technology and Nutrition, New Technologies in Aquaculture* (pp. 822-844). Woodhead Publishing https://doi.org/10.1533/9781845696474.5.822

Jones CM, Anh TL, & Priyambodo B. (2019). Lobster Aquaculture Development in Vietnam and Indonesia. In EV Radhakrishnan, B Phillips, & G Achamveetil (Eds.), *Lobster: Biology, Fisheries and Aquaculture* (pp. 541-570). Springer. https://doi.org/https://doi.org/10.1007/978-981-32-9094-5_13

Jucker C, Erba D, Leonardi M, Lupi D, & Savoldelli S. (2017). Assessment of Vegetable and Fruit Substrates as Potential Rearing Media for *Hermetia illucens* (Diptera: Stratiomyidae) Larvae. *Environ. Entomol.*, *46*. https://doi.org/10.1093/ee/nvx154

Julita U, Fitri L, Putra R, & Permana A. (2019). Survival and Reproductive Value of Hermetia illucens (Diptera: Stratiomyidae) on Vegetable and Fruits Waste Rearing Substrate. *Journal of Physics: Conference Series*, *1245*, 012002. https://doi.org/10.1088/1742-6596/1245/1/012002

Kabir KA, Verdegem MCJ, Verreth JAJ, Phillips MJ, & Schrama JW. (2019). Effect of dietary protein to energy ratio, stocking density and feeding level on performance of Nile tilapia in pond aquaculture. *Aquaculture*, *511*, 634200. https://doi.org/10.1016/j.aquaculture.2019.06.014

Khan KU, Tellechea Rodrigues A, Menegasso Mansano C, de Almeida D, Kazue Sakomura N, de Souza Romaneli R, Torres do Nascimento T, & Kochenborger Fernandes J. (2019). Dietary protein quality and proper protein to energy ratios: a bioeconomic approach in aquaculture feeding practices. *Lat. Am. J. Aquat. Res.*, *47*(2), 232-239. https://doi.org/10.3856/vol47-issue2-fulltext-3

Koshio S, Teshima S-i, Kanazawa A, & Watase T. (1993). The effect of dietary protein content on growth, digestion efficiency and nitrogen excretion of juvenile kuruma prawns, *Penaeus japonicus*. *Aquaculture*, *113*(1), 101-114. https://doi.org/10.1016/0044-8486(93)90344-X

Kropielnicka-Kruk K, Fitzgibbon Q, Codabaccus M, Trotter A, Giosio D, Carter C, & Smith G. (2022). The Effect of Feed Frequency on Growth, Survival and Behaviour of Juvenile Spiny Lobster (*Panulirus ornatus*). *Animals*, *12*, 2241. https://doi.org/10.3390/ani12172241

Kropielnicka-Kruk K, Trotter AJ, Fitzgibbon QP, Smith GG, & Carter CG. (2019). The effect of conspecific interaction on survival, growth and feeding behaviour of early juvenile tropical spiny lobster *Panulirus ornatus*. *Aquaculture*, *510*, 234-247. https://doi.org/10.1016/j.aquaculture.2019.05.017

Kurnia A, Yusnaini Y, H.Muskita W, Astuti O, & Hamzah M. (2017). Replacement of Fish Meal with Fish Head Meal in the Diet on the Growth and Feed Efficiency of Spiny Lobster, *Panulirus ornatus* Under Reared in Sea Net Cage. *The International Journal of Engineering and Science*, *06*, 34-38. https://doi.org/10.9790/1813-0601033438

Lan PTP, Quan NH, Ngoan LD, Hong TTT, & Tram NDQ. (2022). Amino acid and fatty acid compositions of black soldier fly larvae (*Hermetia illucens*) fed by

Tofu by-products in Viet Nam. *Livestock Research for Rural Development*, *34*(84). http://www.lrrd.org/lrrd34/10/3484nguy.html

Latorres JM, Rios DG, Saggiomo G, Wasielesky W, Jr., & Prentice-Hernandez C. (2018). Functional and antioxidant properties of protein hydrolysates obtained from white shrimp (*Litopenaeus vannamei*). *J. Food Sci. Technol.*, *55*(2), 721-729. https://doi.org/10.1007/s13197-017-2983-z

Lellis W. (1992). A Standard Reference Diet for Crustacean Nutrition Research VI. Response of Postlarval Stages of the Caribbean King Crab *Mithrax spinosissimus* and the Spiny Lobster *Panulirus argus*. *J. World Aquacult. Soc.*, *23*, 1-7. https://doi.org/10.1111/j.1749-7345.1992.tb00744.x

Lemos D, & Weissman D. (2021). Moulting in the grow-out of farmed shrimp: a review. *Reviews in Aquaculture*, *13*(1), 5-17. https://doi.org/10.1111/raq.12461

Li S, Ji H, Zhang B, Zhou J, & Yu H. (2017). Defatted black soldier fly ( *Hermetia illucens* ) larvae meal in diets for juvenile Jian carp ( *Cyprinus carpio* var. Jian): Growth performance, antioxidant enzyme activities, digestive enzyme activities, intestine and hepatopancreas histological structure. *Aquaculture*, *477*, 62-70. https://doi.org/10.1016/j.aquaculture.2017.04.015

Li Y, Bordinhon AM, Allen Davis D, Zhang W, & Zhu X. (2012). Protein: energy ratio in practical diets for Nile tilapia *Oreochromis niloticus*. *Aquacult. Int.*, *21*(5), 1109-1119. https://doi.org/10.1007/s10499-012-9616-3

Li Y, Kortner TM, Chikwati EM, Belghit I, Lock E-J, & Krogdahl Å. (2020). Total replacement of fish meal with black soldier fly (*Hermetia illucens*) larvae meal does not compromise the gut health of Atlantic salmon (*Salmo salar*). *Aquaculture*, *520*, 734967. https://doi.org/10.1016/j.aquaculture.2020.734967

Lu S, Taethaisong N, Meethip W, Surakhunthod J, Sinpru B, Sroichak T, Archa P, Sorasak T, Paengkoum S, Purba R, & Paengkoum P. (2022). Nutritional Composition of Black Soldier Fly Larvae (*Hermetia illucens* L.) and Its Potential Uses as Alternative Protein Sources in Animal Diets: A Review. *Insects*, *13*, 831. https://doi.org/10.3390/insects13090831

Magalhães R, Sánchez-López A, Leal RS, Martínez-Llorens S, Oliva-Teles A, & Peres H. (2017). Black soldier fly ( *Hermetia illucens* ) pre-pupae meal as a fish meal replacement in diets for European seabass ( *Dicentrarchus labrax* ). *Aquaculture*, *476*, 79-85. https://doi.org/10.1016/j.aquaculture.2017.04.021

Makkar HPS, Tran G, Heuzé V, & Ankers P. (2014). State-of-the-art on use of insects as animal feed. *Anim. Feed Sci. Technol.*, *197*, 1-33. 10.1016/j.anifeedsci.2014.07.008

Manambrakat V, & Kumar TS. (2010). Moulting and growth in communal and individual rearing of the spiny lobster, *Panulirus homarus*. *J. Mar. Biol. Assoc. India*, *52*, 274-281.

Manan H, Amin-Safwan A, Shakri IW, Kasan NA, & Ikhwanuddin M. (2022). Histology and External Morphological Assessment on Ovarian Maturation Stages of Wild Female Banana Shrimp, *Penaeus merguiensis* (de Man, 1888) for Broodstock Selection Purpose. *Tropical life sciences research*, *33*(1), 91-103. https://doi.org/10.21315/tlsr2022.33.1.6

Marchese G, Fitzgibbon Q, Trotter A, Carter C, Jones C, & Smith G. (2018). The influence of flesh ingredients format and krill meal on growth and feeding behaviour of juvenile tropical spiny lobster *Panulirus ornatus*. *Aquaculture*, *499*. https://doi.org/10.1016/j.aquaculture.2018.09.019

Méndez-Martínez Y, García-Guerrero MU, Arcos-Ortega FG, Martínez-Córdova LR, Yamasaki-Granados S, Pérez-Rodríguez JC, & Cortés-Jacinto E. (2018). Effect of different ratios of dietary protein-energy on growth, body proximal composition, digestive enzyme activity, and hepatopancreas histology in *Macrobrachium americanum* (Bate, 1868) prawn juveniles. *Aquaculture*, *485*, 1-11. https://doi.org/10.1016/j.aquaculture.2017.11.012

Miao S, Zhao C, Jinyu Z, Hu J, Dong X, & Sun L. (2018). Dietary soybean meal affects intestinal homoeostasis by altering the microbiota, morphology and inflammatory cytokine gene expression in northern snakehead. *Sci. Rep.*, *8*. https://doi.org/10.1038/s41598-017-18430-7

Mohamad SJ, Zailudin AF, Zakariah MI, Yusoff NAH, & Hassan M. (2021). Evaluation of Carboxymethyl Cellulose (CMC) Binder and Squid Oil Attractant in the Formulation of *Litopenaeus vannamei* Diet. *Jurnal Imiah Perikanan dan Kelautan*, *13*(2), 279-287. https://doi.org/10.20473/jipk.v13i2.24428

Mohan K, Rajan DK, Muralisankar T, Ganesan AR, Sathishkumar P, & Revathi N. (2022). Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: A review of past and future needs. *Aquaculture*, *553*, 738095. https://doi.org/10.1016/j.aquaculture.2022.738095

Moreira TFM, Gonçalves OH, Leimann FV, & Ribeiro RP. (2023). Fish Protein Hydrolysates: Bioactive Properties, Encapsulation and New Technologies for Enhancing Peptides Bioavailability. *Curr. Pharm. Des.*, *29*(11), 824-836. https://doi.org/10.2174/1381612829666230110141811

Motte C, Rios A, Lefebvre T, Do H, Henry M, & Jintasataporn O. (2019). Replacing Fish Meal with Defatted Insect Meal (Yellow Mealworm *Tenebrio molitor*) Improves the Growth and Immunity of Pacific White Shrimp *(Litopenaeus vannamei*). *Animals*, *9*, 258. http://doi.org/10.3390/ani9050258

Mulyasari M, Sunarno M, & Suryaningrum L. (2018). Isolation, Characterization, and Identification of Proteolytic Bacteria to Improve Protein Digestibility of Fish Feed Ingredients *Indonesian Aquaculture Journal*, *13*, 83-93. https://doi.org/10.15578/iaj.13.2.2018.xx-xx

Nankervis L, & Jones C. (2022). Recent advances and future directions in practical diet formulation and adoption in tropical Palinurid lobster aquaculture. *Reviews in Aquaculture*. https://doi.org/10.1111/raq.12675

Nelson MM, Bruce MP, Nichols PD, Jeffs AG, & Phleger CF. (2006). Nutrition of Wild and Cultured Lobsters. *Lobsters: Biology, Management, Aquaculture and Fisheries*, 205-230. https://doi.org/doi:10.1002/9780470995969.ch6

Nguyen HTM, Pérez-Gálvez R, & Bergé JP. (2012). Effect of diets containing tuna head hydrolysates on the survival and growth of shrimp *Penaeus vannamei*.

*Aquaculture*, *324-325*, 127-134. https://doi.org/10.1016/j.aquaculture.2011.11.014

Nguyen Quang L, Tran Nguyen N, Kieu Thi H, Ngo Thi Huong G, & Nguyen Van H. (2015). Nutritional Characteristics and Feeding of Rabbitfish (Siganus guttatus) in Tam Giang-Cau Hai Lagoon Systems. *Journal of Agricultural Science and Technology A*, *5*(12). 10.17265/2161-6256/2015.12.015

Nielsen SS. (2017). Moisture Content Determination. In SS Nielsen (Ed.), *Food Analysis Laboratory Manual* (pp. 105-115). Springer International Publishing. https://doi.org/https://doi.org/10.1007/978-3-319-44127-6_11

Niu J, Zhang Y-Q, Liu Y-J, Tian L-X, Lin H-Z, Chen X, Yang H-J, & Liang G-Y. (2014). Effects of graded replacement of fish meal by fish protein hydrolysate on growth performance of early post-larval Pacific white shrimp (*Litopenaeus vannamei*, Boone). *Journal of Applied Animal Research*, *42*(1), 6-15. https://doi.org/10.1080/09712119.2013.795897

NRC. (1993). *Nutrient Requirements of Fish*. National Academy Press. https://books.google.com.au/books?id=J9KX77YEYikC

Nugroho RA, & Fotedar R. (2015). Effects of dietary organic selenium on immune responses, total selenium accumulation and digestive system health of marron, *Cherax cainii* (Austin, 2002). *Aquacult. Res.*, *46*(7), 1657-1667. https://doi.org/10.1111/are.12320

Pan MV, Traifalgar RFM, Ferriols VMEN, Laureta LV, & Peralta JP. (2022). Efficacy of squid by-product hydrolysate supplementation on the growth performance, digestive enzyme activities and muscle growth-related genes expression of *Penaeus monodon* fed on plant protein-based diet. *Cogent Food & Agriculture*, *8*(1), 2103067. https://doi.org/10.1080/23311932.2022.2103067

Panini RL, Freitas LEL, Guimarães AM, Rios C, da Silva MFO, Vieira FN, Fracalossi DM, Samuels RI, Prudêncio ES, Silva CP, & Amboni RDMC. (2017). Potential use of mealworms as an alternative protein source for Pacific white shrimp: Digestibility and performance. *Aquaculture*, *473*, 115-120. https://doi.org/10.1016/j.aquaculture.2017.02.008

Paul B, Chanda S, Das S, Singh P, & Giri S. (2014). Mineral Assay in Atomic Absorption Spectroscopy. *The Beats of Natural Sciences*, *1*, 1-17. Retrieved 12/01, from http://www.sncwgs.ac.in/wp-content/uploads/2015/01/Article1.pdf

Perera E, Fraga I, Carrillo O, Díaz-Iglesias E, Cruz R, Báez M, & Galich GS. (2005). Evaluation of practical diets for the Caribbean spiny lobster *Panulirus argus* (Latreille, 1804): effects of protein sources on substrate metabolism and digestive proteases. *Aquaculture*, *244*(1), 251-262. https://doi.org/10.1016/j.aquaculture.2004.11.022

Perera E, Moyano Fj Fau - Díaz M, Díaz M Fau - Perdomo-Morales R, Perdomo-Morales R Fau - Montero-Alejo V, Montero-Alejo V Fau - Rodriguez-Viera L, Rodriguez-Viera L Fau - Alonso E, Alonso E Fau - Carrillo O, Carrillo O Fau - Galich GS, & Galich GS. (2008). Changes in digestive enzymes through developmental and molt stages in the spiny lobster, *Panulirus argus*.

*Comparative Biochesmistry and Physiology, Part B*, *151*(1096-4959 (Print)). 10.1016/j.cbpb.2008.07.005

Perera E, & Simon C. (2014). Digestive physiology of spiny lobsters: Implications for formulated diet development. *Reviews in Aquaculture*, *6*, 1-19. https://doi.org/10.1111/raq.12066

Petersen EH, & Phuong TH. (2010). Tropical spiny lobster (*Panulirus ornatus*) farming in Vietnam - bioeconomics and perceived constraints to development. *Aquacult. Res.*, *41*(10). http://europepmc.org/abstract/AGR/IND44427865

Petersen L, Jones C, & Priyambodo B. (2015). Bio-economics of spiny lobster farming in Indonesia. In CM Jones (Ed.), *Proceedings of the International Lobster Aquaculture Symposium* (Vol. 145, pp. 285). ACIAR

Phillips B, & Matsuda H. (2011). A Global Review of Spiny Lobster Aquaculture. *Recent Advances and New Species in Aquaculture*, 22-84. https://doi.org/10.1002/9781444341775.ch2

Prayitno S, B. , Rusdi I, Giri A, & Haryanti. (2020). Post puerulus of scalloped spiny lobster, *Panulirus homarus* (Linnaeus 1758) rearing in floating net cage with different artificial diet. *IOP Conference Series: Earth and Environmental Science*, *521*, 012005. https://doi.org/10.1088/1755-1315/521/1/012005

Priyadarshana K, Ruwandeepika C, Naveenan M, Magamage M, & Ruwandeepika D. (2021). Substitution of Fishmeal with Black Soldier Fly *Hermetia illucens* Linnaeus, 1758 Larvae in Finfish Aquaculture -A Review. *Asian Fish. Sci.*, *34*. https://doi.org/10.33997/j.afs.2021.34.2.001

Priyambodo B. (2018). *The development of spiny lobster aquaculture in Indonesia through the enhancement of puerulus catch and technology transfer* The University of New South Wales]. New South Wales.

Priyambodo B, Jones C, & Sammut J. (2015). The effect of trap type and water depth on puerulus settlement in the spiny lobster aquaculture industry in Indonesia. *Aquaculture*, *442*, 132-137. https://doi.org/10.1016/j.aquaculture.2015.02.037

Ptacek M, Sarver S, Childress M, & Herrnkind W. (2001). Molecular phylogeny of the spiny lobster genus Panulirus (Decapoda: Palinuridae). *Mar. Freshwater Res*, *52*, 1037-1047. https://doi.org/10.1071/MF01070

Quinto BPT, Albuquerque JV, Bezerra RS, Peixoto S, & Soares R. (2018). Replacement of fishmeal by two types of fish protein hydrolysate in feed for postlarval shrimp *Litopenaeus vannamei*. *Aquacult. Nutr.*, *24*(2), 768-776. https://doi.org/10.1111/anu.12605

Rabani V, Cheatsazan H, & Davani S. (2019). Proteomics and lipidomics of black soldier fly (Diptera: Stratiomyidae) and blow fly (Diptera: Calliphoridae) Larvae. *Journal of Insect Science*, *19*(3), 1-9. https://doi.org/10.1093/jisesa/iez050

Radhakrishnan EV, Phillips B, & Gopalakrishnan D. (2020). *Lobsters: Biology, Fisheries and Aquaculture*. https://doi.org/https://doi.org/10.1007/978-981-32-9094-5

Radhakrishnan EV, Phillips BF, Lakshmi PS, & Padua S. (2019). Ecology and Global Distribution Pattern of Lobsters. In EV Radhakrishnan, BF Phillips, & G

Achamveetil (Eds.), *Lobsters: Biology, Fisheries and Aquaculture*. Springer Nature. https://doi.org/https://doi.org/10.1007/978-981-32-9094-5_5

Rajamani M, & Manickaraja M. (1997). The spiny lobster resources in the trawling grounds off Tuticorin. *Marine Fisheries Information Service T&E Series*, *148*, 7-9.

Rathinam AMM, Kandasami D, Kizhakudan JK, Leslie VA, & Gandhi AD. (2009). Effect of dietary protein on the growth of spiny lobster *Panulirus homarus* (Linnaeus). *J. Mar. Biol. Assoc. India*, *51*(1), 114-117.

Ratnayake WMN, Hansen SL, & Kennedy MP. (2006). Evaluation of the CP-Sil 88 and SP-2560 GC columns used in the recently approved AOCS official method Ce 1h-05: Determination of cis-, trans-, saturated, monounsaturated, and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC method. *J. Am. Oil Chem. Soc.*, *83*(6), 475-488. https://doi.org/10.1007/s11746-006-1230-y

Ratunil V. (2017). Growth and survival of ornate spiny lobster (*Panulirus ornatus*) in nursery under laboratory condition. *Philippine Journal of Agricultural Economics*, *1*, 16. https://doi.org/10.7719/pjae.v1i1.483

Ravi H, Degrou A, Costil J, Trespeuch C, Chemat F, & Vian M. (2020). Larvae mediated valorization of industrial, agriculture and food wastes: Biorefinery concept through bioconversion, processes, procedures, and products. *Processes*, *8*, 857. https://doi.org/10.3390/pr8070857

Rawski M, Mazurkiewicz J, Kierończyk B, & Józefiak D. (2020). Black Soldier Fly Full-Fat Larvae Meal as an Alternative to Fish Meal and Fish Oil in Siberian Sturgeon Nutrition: The Effects on Physical Properties of the Feed, Animal Growth Performance, and Feed Acceptance and Utilization. *Animals*, *10*(11). https://doi.org/10.3390/ani10112119

Richardson A, Dantas-Lima J, Lefranc M, & Walraven M. (2021). Effect of a black soldier fly ingredient on the growth performance and disease resistance of juvenile pacific white shrimp (*Litopenaeus vannamei*). *Animals (Basel)*, *11*(5). https://doi.org/10.3390/ani11051450

Riddick EW. (2014). Chapter 16. Insect Protein as a Partial Replacement for Fishmeal in the Diets of Juvenile Fish and Crustaceans. In Juan A. Morales-Ramos, M. Guadalupe Rojas, & David I. Shapiro-Ilan (Eds.), *Mass Production of Beneficial Organisms* (pp. 565-582). Academic Press. https://doi.org/https://doi.org/10.1016/b978-0-12-391453-8.00016-9

Ridwanudin A, Fahmi V, & Pratama IS. (2018). Growth of Spiny Lobster *Panulirus homarus* Fed with Moist Diet. *Oseanologi dan Limnologi di Indonesia*, *3*(2), 95. https://doi.org/10.14203/oldi.2018.v3i2.165

Rodríguez-Viera L, & Perera E. (2012). *Panulirus argus* postlarva performance fed with fresh squid. *Rev. Invest. Mar.*, *32*, 9-15.

Rodríguez-Viera L, Perera E, Casuso A, Perdomo-Morales R, Gutierrez O, Scull I, Carrillo O, Martos-Sitcha JA, García-Galano T, & Mancera JM. (2014). A Holistic View of Dietary Carbohydrate Utilization in Lobster: Digestion, Postprandial Nutrient Flux, and Metabolism. *PLoS One*, *9*(9), e108875. https://doi.org/10.1371/journal.pone.0108875

Rodriguez-Viera L, Perera E, Martos-Sitcha JA, Perdomo-Morales R, Casuso A, Montero-Alejo V, Garcia-Galano T, Martinez-Rodriguez G, & Mancera JM. (2016). Molecular, Biochemical, and Dietary Regulation Features of alpha-Amylase in a Carnivorous Crustacean, the Spiny Lobster *Panulirus argus*. *PLoS One*, *11*(7), e0158919. https://doi.org/10.1371/journal.pone.0158919

Rodríguez-Viera L, Perera E, Montero-Alejo V, Perdomo-Morales R, García-Galano T, Martínez-Rodríguez G, & Mancera JM. (2017). Carbohydrates digestion and metabolism in the spiny lobster (*Panulirus argus*): biochemical indication for limited carbohydrate utilization. *PeerJ*, *5*, e3975-e3975. https://doi.org/10.7717/peerj.3975

Sánchez-Muros MJ, Barroso FG, & de Haro C. (2016). Brief Summary of Insect Usage as an Industrial Animal Feed/Feed Ingredient. In Aaron T. Dossey, Juan A. Morales-Ramos, & MG Rojas (Eds.), *Insects as Sustainable Food Ingredients* (pp. 273-309). Academic Press. https://doi.org/10.1016/b978-0-12-802856-8.00010-7

Sang HM, & Fotedar R. (2010). Prebiotic Mannan Oligosaccharide Diet Improves Health Status of the Digestive System of Marron, *Cherax tenuimanus* (Smith 1912). *J. Appl. Aquacult.*, *22*(3), 240-250. 10.1080/10454438.2010.500593

Saputra I, & Fotedar R. (2023). The effect of defatted black soldier fly meal (*Hermetia illucens*) inclusion in the formulated diet on the growth, gene expression, and histopathology of juvenile lobster (*Panulirus ornatus* Fabricius, 1798). *Aquacult. Int.*, 1-19. https://doi.org/10.1007/s10499-023-01151-2

Saputra I, Fotedar R, Gupta SK, Siddik MAB, & Foysal MJ. (2019). Effects of different dietary protein sources on the immunological and physiological responses of marron, *Cherax cainii* (Austin and Ryan, 2002) and its susceptibility to high temperature exposure. *Fish Shellfish Immunol.*, *88*, 567-577. https://doi.org/10.1016/j.fsi.2019.03.012

Saputra I, & Lee Y. (2023). Nutrition composition of commercial full-fat and defatted black soldier fly larvae meal (*Hermetia illucens*) as a potential protein resource for aquafeeds. *Biodiversitas*, *24*(9), 4877-4884. https://doi.org/10.13057/biodiv/d240930

Schiavone A, De Marco M, Martínez S, Dabbou S, Renna M, Madrid J, Hernandez F, Rotolo L, Costa P, Gai F, & Gasco L. (2017). Nutritional value of a partially defatted and a highly defatted black soldier fly larvae (*Hermetia illucens* L.) meal for broiler chickens: apparent nutrient digestibility, apparent metabolizable energy and apparent ileal amino acid digestibility. *Journal of Animal Science and Biotechnology*, *8*(1), 1-9. https://doi.org/10.1186/s40104-017-0181-5

Shanks S, Bahrawi S, Priyambodo B, & Jones C. (2015). Transport and husbandry of postpuerulus tropical spiny lobsters. Proceedings of the International Lobster Aquaculture Symposium, Lombok, Indonesia.

Shearer KD, Maage A, Opstvedt J, & Mundheim H. (1992). Effects of high-ash diets on growth, feed efficiency, and zinc status of juvenile Atlantic salmon (Salmo

salar). *Aquaculture*, *106*(3), 345-355. https://doi.org/10.1016/0044-8486(92)90266-N

Shields J, Wheeler K, & Moss J. (2012). Histological Assessment of the Lobster (*Homarus americanus*) in the "100 Lobsters" Project. *J. Shellfish Res.*, *31*, 439-447. https://doi.org/10.2983/035.031.0204

Shu-Chien AC, Han W-Y, Carter CG, Fitzgibbon QP, Simon CJ, Kuah M-K, Battaglene SC, Codabaccus BM, & Ventura T. (2017). Effect of dietary lipid source on expression of lipid metabolism genes and tissue lipid profile in juvenile spiny lobster *Sagmariasus verreauxi*. *Aquaculture*, *479*, 342-351. https://doi.org/10.1016/j.aquaculture.2017.05.036

Shumo M, Osuga IM, Khamis FM, Tanga CM, Fiaboe KKM, Subramanian S, Ekesi S, van Huis A, & Borgemeister C. (2019). The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Sci. Rep.*, *9*(1), 10110. https://doi.org/10.1038/s41598-019-46603-z

Simon CJ. (2009). Digestive enzyme response to natural and formulated diets in cultured juvenile spiny lobster, *Jasus edwardsii*. *Aquaculture*, *294*(3-4), 271-281. https://doi.org/10.1016/j.aquaculture.2009.06.023

Simon CJ, & Jeffs A. (2011). The effect of dietary carbohydrates on the growth response, digestive gland glycogen and digestive enzyme activities of early spiny lobster juveniles, *Jasus edwardsii*. *Aquacult. Nutr.*, *17*(6), 613-626. https://doi.org/10.1111/j.1365-2095.2011.00861.x

Simon CJ, & Jeffs AG. (2013). The effect of dietary carbohydrate on the appetite revival and glucose metabolism of juveniles of the spiny lobster, *Jasus edwardsii*. *Aquaculture*, *384-387*, 111-118. https://doi.org/10.1016/j.aquaculture.2013.01.003

Slamet B, Rusdi I, Giri A, & Haryanti. (2020a). Post puerulus of scalloped spiny lobster, *Panulirus homarus* (Linnaeus 1758) rearing in floating net cage with different artificial diet. *IOP Conference Series: Earth and Environmental Science*, *521*, 012005. https://doi.org/10.1088/1755-1315/521/1/012005

Slamet B, Rusdi I, Giri A, & Haryanti. (2020b). Post puerulus of scalloped spiny lobster, *Panulirus homarus* (Linnaeus 1758) rearing in floating net cage with different artificial diet. *IOP Conference Series: Earth and Environmental Science*, *521*(1), 012005. https://doi.org/10.1088/1755-1315/521/1/012005

Slamet B, Rusdi I, Giri A, & Haryanti. (2021). Effect of shelter net sizes on growth, survivality, and health of scalloped spiny lobster, *Panulirus homarus* (Linnaeus 1758) reared in fiberglass tank. *IOP Conference Series: Earth and Environmental Science*, *919*(1), 012051. https://doi.org/10.1088/1755-1315/919/1/012051

Smith DM, Williams KC, Irvin S, Barclay M, & Tabrett S. (2003). Development of a pelleted feed for juvenile tropical spiny lobster (*Panulirus ornatus*): response to dietary protein and lipid. *Aquacult. Nutr.*, *9*(4), 231-237. https://doi.org/10.1046/j.1365-2095.2003.00248.x

Smith DM, Williams KC, & Irvin SJ. (2005). Response of the tropical spiny lobster *Panulirus ornatus* to protein content of pelleted feed and to a diet of mussel

flesh. *Aquaculture nutrition.*, *11*(3), 209-217. https://doi.org/10.1111/j.1365-2095.2005.00344.x

Smith G, Salmon M, Kenway M, & Hall M. (2009). Description of the larval morphology of captive reared *Panulirus ornatus* spiny lobsters, benchmarked against wild-caught specimens. *Aquaculture*, *295*(1), 76-88. https://doi.org/10.1016/j.aquaculture.2009.06.030

Soetemans L, Uyttebroek M, & Bastiaens L. (2020). Characteristics of chitin extracted from black soldier fly in different life stages. *Int. J. Biol. Macromol.*, *165*, 3206-3214. https://doi.org/10.1016/j.ijbiomac.2020.11.041

Spranghers T, Ottoboni M, Klootwijk C, Ovyn A, Deboosere S, De Meulenaer B, Michiels J, Eeckhout M, De Clercq P, & De Smet S. (2017). Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. *J. Sci. Food Agric.*, *97*(8), 2594-2600. https://doi.org/10.1002/jsfa.8081

Sreeram MP, & Menon N. (2005). Histopathological changes in the hepatopancreas of the penaeid shrimp Metapenaeus dobsoni exposed to petroleum hydrocarbons. *Journal of Marine Biological Association of India*, *47*.

Su J, Gong Y, Cao S, Lu F, Han D, Liu H, Jin J, Yang Y, Zhu X, & Xie S. (2017). Effects of dietary *Tenebrio molitor* meal on the growth performance, immune response and disease resistance of yellow catfish (*Pelteobagrus fulvidraco*). *Fish Shellfish Immunol.*, *69*, 59-66. https://doi.org/10.1016/j.fsi.2017.08.008

Sudaryono A. (2001). Pellet water stability studies on lupin meal based shrimp (Penaeus monodon) aquaculture feeds: comparison of lupin meal with other dietary protein sources. *Journal of Coastal Development*, *4*(3), 129-140.

Sudewi, Giri NA, Slamet B, & Haryanti. (2023). Growth at molt of sub-adult scalloped spiny lobster Panulirus homarus reared in tanks. *IOP Conference Series: Earth and Environmental Science*, *1221*(1), 012015. 10.1088/1755-1315/1221/1/012015

Sudtongkong C, Thongprajukaew K, & Saekhow S. (2020). Optimal Dietary Protein Requirement for Juvenile Sesarmid Crab (*Episesarma singaporense*). *Animals*, *10*(6), 998. https://doi.org/10.3390/ani10060998

Suma AY, Nandi SK, Abdul Kari Z, Goh KW, Wei LS, Tahiluddin AB, Seguin P, Herault M, Al Mamun A, Téllez-Isaías G, & Anamul Kabir M. (2023). Beneficial effects of graded levels of fish protein hydrolysate (FPH) on the growth performance, blood biochemistry, liver and intestinal health, economics efficiency, and disease resistance to *Aeromonas hydrophila* of pabda (*Ompok pabda*) fingerling. *Fishes*, *8*(3). https://doi.org/10.3390/fishes8030147

Syafrizal, Jones CM, Permana IG, & Utomo NBP. (2018). Effect of feeding frequency on survival and growth of juvenile spiny lobster Panulirus versicolor in Indonesia. *AACL Bioflux*, *11*(5).

Taynor MCJ. (2013). *Evaluation of gross Protein/Energy Ratios for Adult Florida Pompano, Trachinotus carolinus, Fed Practical diets, in relation to growth, feed utilization, and nitrogen retention* University of Miami]. Coral GAbles, Florida.

Teshima S-i. (1998). Nutrition of *Penaeus japonicus*. *Rev. Fish. Sci.*, *6*(1-2), 97-111. https://doi.org/10.1080/10641269891314221

Thévenot A, Rivera JL, Wilfart A, Maillard F, Hassouna M, Senga-Kiesse T, Le Féon S, & Aubin J. (2018). Mealworm meal for animal feed: Environmental assessment and sensitivity analysis to guide future prospects. *Journal of Cleaner Production*, *170*, 1260-1267. https://doi.org/10.1016/j.jclepro.2017.09.054

Tietz NW, & Fiereck EA. (1966). A specific method for serum lipase determination. *Clin. Chim. Acta*, *13*(3), 352-358. 10.1016/0009-8981(66)90215-4

Tuan LAUoF, Nha Trang, Khan Hoa (Vietnam)) Nho, N.T., & Hambrey J. (2000). Status of cage mariculture in Vietnam.

Uy T, Fitzgibbon QP, Codabaccus BM, & Smith GG. (2023). Thermal physiology of tropical rock lobster (*Panulirus ornatus*); defining physiological constraints to high temperature tolerance. *Aquaculture*, *569*, 739357. https://doi.org/10.1016/j.aquaculture.2023.739357

Venero JA, Davis DA, & Rouse DB. (2008). Effect of the Dietary Ratio of Digestible Energy to Crude Protein on Growth and Feed Conversion in Juvenile Pacific White Shrimp *Litopenaeus vannamei* under Similar Levels of Daily Protein Consumption. *N. Am. J. Aquacult.*, *70*(1), 43-49. https://doi.org/10.1577/a07-012.1

Vijayakumaran M, Anbarasu M, & Kumar TS. (2010). Moulting and growth in communal and individual rearing of the spiny lobster, *Panulirus homarus*. *J. Mar. Biol. Assoc. India*, *52*, 274-281.

Vijayakumaran M, Venkatesan R, Murugan TS, Kumar TS, Jha DK, Remany MC, Thilakam JML, Jahan SS, Dharani G, Kathiroli S, & Selvan K. (2009). Farming of spiny lobsters in sea cages in India. *N. Z. J. Mar. Freshwat. Res.*, *43*(2), 623-634. https://doi.org/10.1080/00288330909510028

Vijayakumaran V. (2004). Nutritional Requirements and Feeds of Spiny Lobsters. Proceedings of MBR 2004, India.

Vogt G. (2019). Functional cytology of the hepatopancreas of decapod crustaceans. *J. Morphol.*, *280*(9), 1405-1444. https://doi.org/10.1002/jmor.21040

Wang G, Peng K, Hu J, Yi C, Chen X, Wu H, & Huang Y. (2019). Evaluation of defatted black soldier fly (*Hermetia illucens L.*) larvae meal as an alternative protein ingredient for juvenile Japanese seabass (*Lateolabrax japonicus*) diets. *Aquaculture*, *507*, 144-154. https://doi.org/10.1016/j.aquaculture.2019.04.023

Wang KH-C, Tseng CW, Lin HY, Chen IT, Chen YH, Chen YM, Chen TY, & Yang HL. (2010). RNAi knock-down of the Litopenaeus vannamei Toll gene (LvToll) significantly increases mortality and reduces bacterial clearance after challenge with Vibrio harveyi. *Dev. Comp. Immunol.*, *34*(1), 49-58. https://doi.org/10.1016/j.dci.2009.08.003

Ward LR. (2005). *Protein and lipid nutrition of juvenile southern rock lobster, Jasus edwardsii (Hutton)* University of Tasmania]. Tasmania.

Ward LR, Carter C, Crear BJ, & Smith DM. (2003). Optimal dietary protein level for juvenile southern rock lobster, *Jasus edwardsii*, at two lipid levels. *Aquaculture*, *217*, 483-500. https://doi.org/10.1016/S0044-8486(02)00258-2

Ward LR, Carter CG, & Crear BJ. (2003). Apparent digestibility of potential ingredients as protein sources in formulated feeds for the southern rock lobster Jasus edwardsii. In K Wiliams (Ed.), *Rock Lobster Enhancement & Aquaculture Subprogram: The Nutrition of Juvenile and Adult Lobsters to Optimise Survival, Growth and Condition*. ACIAR.

Weththasinghe P, Hansen JØ, Nøkland D, Lagos L, Rawski M, & Øverland M. (2021). Full-fat black soldier fly larvae (*Hermetia illucen*s) meal and paste in extruded diets for Atlantic salmon (*Salmo salar*): Effect on physical pellet quality, nutrient digestibility, nutrient utilization and growth performances. *Aquaculture*, *530*, 735785. https://doi.org/10.1016/j.aquaculture.2020.735785

Williams KC. (2004). *Rock Lobster Enhancement and Aquaculture Subprogram: The Nutrition of Juvenile and Adult Lobsters to Optimize Survival, Growth and Condition* (Final report of Project 2000/212 to Fisheries Reasearch and Development Corporation, Issue.

Williams KC. (2007). Nutritional requirements and feeds development for post-larval spiny lobster: A review. *Aquaculture*, *263*(1), 1-14. https://doi.org/10.1016/j.aquaculture.2006.10.019

Williams KC. (2009). Nutritional reuirements of juvenile Panulirus ornatus lobster. In KC Williams (Ed.), *Spiny lobster aquaculture in the Asia-Pacific region* (Vol. 132, pp. 162). Australian Centre for International Agricultural Research.

Woolley LD, Jones CL, & Britz PJ. (2010). Effect of dietary protein to energy ratio on growth and nitrogenous waste production of cultured dusky kob *Argyrosomus japonicus*. *Afr. J. Mar. Sci.*, *32*(3), 625-631. https://doi.org/10.2989/1814232x.2010.538164

Xia J, Ge C, & Yao H. (2021). Antimicrobial Peptides from Black Soldier Fly (*Hermetia illucens*) as Potential Antimicrobial Factors Representing an Alternative to Antibiotics in Livestock Farming. *Animals (Basel)*, *11*(7), 1-16. https://doi.org/10.3390/ani11071937

Xing S, Liang X, Zhang X, Oliva-Teles A, Peres H, Li M, Wang H, Mai K, Kaushik SJ, & Xue M. (2023). Essential amino acid requirements of fish and crustaceans, a meta-analysis. *Reviews in Aquaculture*, *n/a*(n/a). https://doi.org/10.1111/raq.12886

Xu W-N, Liu W-B, Shen M-f, Li G-F, Wang Y, & Zhang W-w. (2013). Effect of different dietary protein and lipid levels on growth performance, body composition of juvenile red swamp crayfish (*Procambarus clarkii*) [journal article]. *Aquacult. Int.*, *21*(3), 687-697. https://doi.org/10.1007/s10499-012-9603-8

Xu X, Ji H, Belghit I, & Sun J. (2020). Black soldier fly larvae as a better lipid source than yellow mealworm or silkworm oils for juvenile mirror carp (*Cyprinus carpio* var. specularis). *Aquaculture*, *527*, 735453. https://doi.org/10.1016/j.aquaculture.2020.735453

Yildirim-Aksoy M, Eljack R, Schrimsher C, & Beck BH. (2020). Use of dietary frass from black soldier fly larvae, *Hermetia illucens*, in hybrid tilapia (Nile x Mozambique, *Oreocromis niloticus* x *O. mozambique*) diets improves growth

and resistance to bacterial diseases. *Aquaculture Reports*, *17*, 100373. https://doi.org/10.1016/j.aqrep.2020.100373

Zha H, Jones B, Lewis G, Dong Y, & Jeffs A. (2018). Pathology of tail fan necrosis in the spiny lobster, Jasus edwardsii. *J. Invertebr. Pathol.*, *154*, 5-11. https://doi.org/10.1016/j.jip.2018.03.010

Zhang J, Liu Y, Tian L, Yang H, Liang G, & Xu D. (2012). Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.*, *33*(4), 1027-1032. 10.1016/j.fsi.2012.05.001

Zhang NN, Ma QQ, Fan WJ, Xing Q, Zhao YL, Chen LQ, Ye JY, Zhang ML, & Du ZY. (2017). Effects of the dietary protein to energy ratio on growth, feed utilization and body composition in *Macrobrachium nipponense*. *Aquacult. Nutr.*, *23*(2), 313-321. https://doi.org/10.1111/anu.12395

Zhou Y, Thirumurugan R, Wang Q, Lee CM, & Davis DA. (2016). Use of dry hydrolysate from squid and scallop product supplement in plant based practical diets for Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture*, *465*, 53-59. https://doi.org/10.1016/j.aquaculture.2016.08.028

Zozo B, Wicht MM, Mshayisa VV, & van Wyk J. (2022). The nutritional quality and structural analysis of black soldier fly larvae flour before and after defatting. *Insects*, *13*(2), 1-10. https://doi.org/10.3390/insects13020168

Zulkifli N, Seok-Kian AY, Seng LL, Mustafa S, Kim YS, & Shapawi R. (2022). Nutritional value of black soldier fly (*Hermetia illucens*) larvae processed by different methods. *PLoS One*, *17*(2), e0263924. https://doi.org/10.1371/journal.pone.0263924

*Every reasonable effort has been made to acknowledge the owners of copyright material. I would be pleased to hear from any copyright owner who has been omitted or incorrectly acknowledged.*

## APPENDIX A

### The current Publication from the Thesis

Thesis title: **Physiological changes of post-puerulii spiny lobster (*Panulirus ornatus* Fabricius, 1798) fed low protein to energy ratio diets supplemented with black soldier fly (*Hermetia illucens*) meal**

| No. | Thesis chapter | Publication status |
|---|---|---|
| 1. | Investigating the effect of various dietary protein to energy ratios on juvenile spiny lobsters, *Panulirus ornatus* (Fabricius, 1798) | This Chapter is published in Annals of Animal Sciences https://doi.org/10.2478/aoas-2024-0023 (**Q2**) |
| 2. | Nutritive composition of commercial full fat and defatted black soldier fly larvae meal (*Hermetia illucens*) as a potential protein source for aquafeeds | This Chapter is published in Biodiversitas. 24 (9), 2023. 4899-4884. https://doi.org/10.13057/biodiv/d240930 (**Q3**) |
| 3. | The effect of defatted black soldier fly (*Hermetia illucens*) meal inclusion on the physiology, hepatopancreas histology and immunology-relatives gene of juvenile lobster (*Panulirus ornatus*) | This Chapter is published in *Aquaculture International* (2023). https://doi.org/10.1007/s10499-023-01151-2 (**Q2**) |
| 4. | The effect of supplementation of defatted, full fat black soldier fly and fish protein hydrolysate on the physiology and immunology-relatives gene of juvenile lobster (*Panulirus ornatus*) | This Chapter is published in Aquaculture Nutrition (**Q2**) |