Faculty of Engineering and Science

Evaluating protein to energy ratios on the growth, survival, and immune response of juvenile Giant trevally (*Caranx ignobilis* Forsskal, 1775) fed various fish hydrolysates as fishmeal supplementation

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DECLARATION

I declare that the thesis does not involve any previously published findings of others in any journals. Also, the thesis contains no materials presented in other theses accepted in any universities.

The research presented in the thesis was performed according to the Australian Code of Practice for the care and use of animals for scientific purposes, as approved by the Animal Ethics Committee of Curtin University, Bentley, Australia (Permit Number: ARE2022-15).

Signature

Date: 25 January 2024

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PREAMBLE

There is a shortage of nutritional knowledge on carnivorous marine finfish species in the published literature and the public domain. Giant trevally (*Caranx ignobilis*) is no exception. The species has recently entered as one of the most popular farmed species, however, its culture expansion is getting restricted due to the lack of knowledge in its nutritional requirements.

The thesis includes five chapters. Chapter 1 briefly overviews the use of fish protein hydrolysate (FPH) and meat and bone meal (MBM) as fishmeal replacements in aquafeed. The chapter presents the current issues related to the nutrition of juvenile giant trevally *(Caranx ignobilis* Forsskal, 1775). Based on the current research gaps, the chapter highlights the aims, objects, and significance of the research.

Chapter 2 includes a literature review of biology, aquaculture, and nutritional research in giant trevally. The information on the effects of fishmeal replacement with FPH and MBM on growth, survival, biological and immunological responses are described in this chapter. Attempts are made to critically present the existing knowledge on the keywords used in this research.

Materials and methods are detailed in Chapter 3. The chapter describes diet preparations, fish rearing, sample collection and data analysis of three experiments, including (1) the effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile giant trevally, (2) the effects of dietary supplementation of fish protein hydrolysate performance, feed utilization, body composition, and haematology of juvenile giant, and (3) whether shrimp hydrolysate improve the efficacy of meat and bone meal diet in juvenile giant trevally?

Chapter 4 presents the results of all experiments. Chapter 5 summarises and discusses the results of the research. The chapter discusses the results of all feeding trials and evaluates the present results with the previous studies on protein and lipid requirement with corresponding P/E ratio, the fishmeal replacement with FPH and MBM in aquafeed. The relationship between the present results and commercial farming is also evaluated. Also, chapter 5 provides the main conclusions, limitations, and recommendations for future research.

ABSTRACT

A series of three feeding experiments was conducted to determine optimum protein and lipid levels with corresponding protein to energy (P/E) ratios in juvenile giant trevally *(Caranx ignobilis* Forsskal, 1775), and its growth performance, physiological and immune responses when fed diets including FPH and MBM as protein sources.

In the first experiment, juvenile giant trevally which were fed six diets comprising of three crude protein levels of 42%, 47%, 52% and two crude lipid levels of 10% and 18% in a 3x2 factorial design were assessed for growth, feed utilization and body composition. The second experiment was conducted to investigate how to fish protein hydrolysate (FPH) supplementation affects the growth, feed utilisation, body composition and haematology of juvenile giant trevally. Seven isonitrogenous (52% protein) and isocaloric diets (10% lipid) were formulated, wherein shrimp hydrolysate (SH) and tuna hydrolysate (TH) were used to replace fishmeal at inclusion levels of 0 (control), 30, 60 and 90 g/kg. The third experiment with six treatments in a 3x2 factorial design was conducted to investigate whether shrimp hydrolysate improved the efficacy of the meat and bone meal (MBM) diet replacing fishmeal protein in juvenile giant trevally. A fishmeal-based diet was used as a control, and two low fishmeal diets were prepared by replacing 25% and 50% of protein from fishmeal with MBM. The efficacy of these two fishmeal protein - replaced diets were tested by including another three dietary treatments supplemented with 45 g/kg of SH. Each diet in each experiment was fed to a triplicated group of juvenile giant trevally for eight weeks in a tank-based system.

The results showed that the growth performance and effective feed utilisation were the highest ($P < 0.05$) when juveniles were fed a diet containing 52% protein and 10% lipid with a P/E of 24.63 g/MJ. Significantly increased levels of amino acids in the fish body, including alanine, aspartic acid, glutamine, serine, leucine, threonine, valanine, arginine, were observed in fish fed with 52% protein. Increasing dietary lipid from 10% to 18% led to reduced levels of alanine, glutamine, leucine, threonine, valine and arginine in the fish. The results also showed higher ($P < 0.05$) specific growth rate and feed utilisation in fish fed 30-60 g/kg shrimp hydrolysate (SH) and tuna hydrolysate (TH) than those fed the control diet. 90 g/kg TH caused the deposition of lipid droplets

in the hepatocyte, a sign of liver damage. The elevated serum protein was seen in fish in the control diet, 30-60 g/kg SH diet, 30 g/kg TH diet, demonstrating that these diets were beneficial for the innate immune response in giant trevally. Moreover, no differences were observed in growth and feed utilisation among fish fed 25% and 50% MBM protein diets with SH supplementation and the control. The goblet cell density in the intestine increased in fish fed diets supplemented with SH. The growth of giant trevally was improved by (i) 52% protein and 10% lipid with a P/E ratio of 24.63 g/MJ in diets, (ii) dietary supplemented TH or SH levels of 30-60 g/kg, and (iii) the supplementation of 45 g/kg SH in the diet with protein sources from FM and MBM. Overall, FPH at moderate dietary inclusions had beneficial effects on the growth of juvenile giant trevally fed low fishmeal diets.

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LIST OF ABBREVIATIONS

FPH	Fish protein hydrolysate
MBM	Meat and bone meal
SH	Shrimp hydrolysate
TH	Tuna hydrolysate
CF	Condition factor
FCR	Feed conversion ratio
Hb	Haemoglobin
HSI	Hepatosomatic index
VSI	Viscerasomatic index
SFA	Saturated fatty acids
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
HUFA	Highly unsaturated fatty acids
GCs	Goblet cell number
SD	Standard deviation
SE	Standard error
SEM	Standard error of the mean

LIST OF COMMON AND SCIENTIFIC NAMES

Giant trevally	*Caranx ignobilis*
Blue trevally	*Caranx melampygus*
Atlantic salmon	*Salmo salar*
Atlantic cod	*Gadus morhua*
Rainbow trout	*Oncorhynchus mykiss*
Milkfish	*Chanos chanos*
Gilthead seabream	*Sparus aurata*
Large yellow croaker	*Larimichthys croceus*
European seabass	*Dicentrarchus labrax*
Groupers nei	*Epinephelus* spp.
Orange-spotted grouper	*Epinephelus coioides*
Red-spotted grouper	*Epinephelus akaara*
Malabar grouper	*Epinephelus malabaricus*
Coho salmon	*Oncorhynchus kisutch*
Japanese seabass	*Lateolabrax japonicus*
Golden pompano	*Trachinotus ovatus*
Florida pompano	*Trachinotus carolinus*
Snubnose pompano	*Trachinotus blochii*
Permit	*Trachinotus falcatus*
Japanese amberjack	*Seriola quinqueradiata*
Barramundi/Asian seabass	*Lates calcarifer*
Red drum	*Sciaenops ocellatus*
Bluefin trevally	*Caranx melampygus*
Kingfish	*Seriola lalandi*
White seabream	*Diplodus sargus*

Red seabream	*Pagrus major*
Black sea bass	*Centropristis striata*
Southern flounder	*Paralichthys lethostigma*
Oliver flounder/Japanese flounder	*Paralichthys olivaceus*
Singhi	*Heteropneustes fossilis*
Tongue sole	*Cynoglossus semilaevis*
Black sole	*Solea solea*
Senegalese sole	*Solea senegalensis*
Turbot	*Scophthalmus maximus*
Red-spotted grouper	*Epinephelus akaara*
Chu’s croaker	*Nibea coibor*
Giant croaker	*Nibea japonica*
Blackspotted croaker	*Nibea diacanthus*
Cuneate drum	*Nibea miichthioides*
Yellow drum	*Nibea albiflora*
Cobia	*Rachycentron canadum*
Spotted rose snapper	*Lutjcenus guttatus*
Black catfish	*Rhamdia quelen*
Sutchi catfish	*Pangasius hypophthalmus*
European catfish	*Silurus glanis*
Ussuri catfish	*Pseudobagrus ussuriensis*
Channel catfish	*Ictalurus punctatus*
Bagrid catfish	*Pseudobagrus fulvidraco*
Red-tailed catfish	*Hemibagrus wyckioides*
Pacific bluefin tuna	*Thunnus orientalis*
Putitor mahseer	*Jor putitora*

Gibel carp	*Carassius auratus gibelio*
Grass carp	*Ctenopharygodon idellus*
Persian sturgeon	*Acipenser persicus*
North sea plaice	*Pleuronectes platessa*
Spotted wolffish	*Anarhichas minor*
Korean rockfish	*Sebastes schlegeli*
Pike silverside	*Chirostoma estor*
Striped murrel	*Chana striata*
Lake white fish	*Coregonus clupeaformis*
Blue gouami	*Trichogaster trichopterus*
Meagre	*Argyrosomus regius*
Yellow perch	*Perca flavescens*
Eel	*Anguilla Anguilla*
Atlantic halibut	*Hippoglossus hippoglossus*
Largemouth black bass	*Micropterus salmoides*
Chinese sucker	*Myxocyprinus asiaticus*
Tampaque	*Colossoma macropomum*
Swordtails	*Xiphophorus helleri*

LIST OF PUBLICATIONS

1. Nguyen, M. C., Fotedar, R., & Pham, H. D. (2022). Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile giant trevally (*Caranx ignobilis* Forsskal, 1775). *Aquaculture Research, 53*(17), 6254-6263. doi:https://doi.org/10.1111/are.16098

2. Nguyen, M. C., Fotedar, R., & Pham, H. D. (2023). Can shrimp hydrolysate improve the efficacy of meat and bone meal diet in juvenile giant trevally *Caranx ignobilis*? *Aquaculture International*. doi:10.1007/s10499-023-01250-0

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CHAPTER 1. INTRODUCTION

1.1. Background

Giant trevally *Caranx ignobilis* has been commercially cultured in Vietnam due to its fast growth, high market price, and its adaptability to sea-cage farming (Pham et al., 2021a). Currently, trash fish is still the main feed for giant trevally in Vietnam, owing to the lack of information on its nutritional requirements (Pham et al., 2021a). Thus, the establishment of a balanced formulated feed for giant trevally is the first and essential step towards sustainable farming.

As carnivorous finfish species, giant trevally require high dietary protein that makes feeding expensive, therefore, determining the optimum protein requirement is essential to establish cost-effective and nutritionally adequate diets. Dietary protein provides materials of amino acids for body growth where its content will be primarily used for tissue synthesis if dietary energy from non-protein sources of lipid and carbohydrate meets the energy requirement of fish (Cuzon & Guillaume, 1997). Conversely, inadequate energy from non-protein sources may lead to the catabolism of dietary protein into energy (Cuzon & Guillaume, 1997; Saoud et al., 2012). Excess dietary energy may also increase lipid deposition, compromising the growth of fish (Cuzon & Guillaume, 1997; Ghiasvand et al., 2012). Therefore, the balanced P/E ratio in aqua feeds is the top priority in fish nutritional studies.

Lipids are considered major energy sources for carnivorous fish (Bonvini et al., 2015; Regost et al., 2001; Wang et al., 2017). Lipids can be efficiently used as spare protein. However excess dietary lipids may reduce the growth performance of fish (Bonvini et al., 2015; Regost et al., 2001; Wang et al., 2017). The protein-sparing effect of energy sourced from lipids has been recorded in several fish species (Hung et al., 2017; Kim & Lee, 2005; Ling et al., 2006; Nguyen et al., 2018; Santinha et al., 1999; Wang et al., 2017). An increase in dietary lipids from 6% to 9% could decrease the protein requirement for red-spotted grouper *Epinephelus akaara* from 52% to 48% without affecting the growth of the species (Wang et al., 2017). A similar trend was observed when lipids were reduced from 16% to 12% in 55% protein diets of turbot *Scophthalmus maximus* (Liu et al., 2014). Thus, determining the optimum protein and

lipid requirements with corresponding P/E ratio for each species, is a crucial step towards the development of nutritionally balanced and cost-effective diets. The requirements of protein and lipid have been reported for several marine carnivorous fish species including permit *Trachinotus falcatus* (Nguyen et al., 2018), Asian seabass *Lates calcarifer* (Catacutan & Coloso, 1995), Florida pompano *Trachinotus carolinus* (Riche, 2009), and kingfish *Seriola lalandi* (Booth et al., 2010) but still unknown in juvenile giant trevally. Therefore, the nutritional information in juvenile giant trevally was a priority for this research investigation.

After establishing the most suitable protein and lipid requirement with P/E ratios for the juveniles, it is imperative to know if dietary fishmeal can be partially substituted by other less expensive alternative protein sources. Fishmeal is an excellent protein source for carnivore fish species (Watanabe, 2002), but the increasing demand and high price of fishmeal with the expansion of aquaculture have put pressure on its supply (Khosravi et al., 2015c; Tacon & Metian, 2015; Zheng et al., 2014). Feeding cost accounts for up to 60% of the total aquaculture operational cost, therefore a reduction in fishmeal level in diets may increase the profitability of the operation (Hernández et al., 2016). Efficient use of the less expensive and more sustainable alternative protein source(s), which contain equal or even better quality than fishmeal protein, is crucial for the sustainability of aquaculture.

Fish protein hydrolysate (FPH) is considered a potential alternative protein in aqua-feed (Chalamaiah et al., 2012b). A high quantity of free amino acids and low molecular weight peptides in FPH improve the feed palatability (Ospina-Salazar et al., 2016; Siddik et al., 2018b). Dietary FPH also improves biological nutrient uptake due to its high digestibility and acceptable texture (Gildberg & Stenberg, 2001). FPH at a moderate dietary inclusion levels is beneficial for fish growth and feed utilisation (Bui et al., 2014; Hevroy et al., 2005; Ospina-Salazar et al., 2016; Siddik et al., 2018b; Xu et al., 2016). Amino acid composition and antioxidative peptides of FPH depend on the raw materials used, the nature of the enzymes used, and the protocols of the hydrolysis procedure (Klompong et al., 2009). Tuna hydrolysate (TH) is a favoured source of essential amino acids as its dietary inclusion resulted in the enhanced growth of oliver flounder *Paralichthys olivaceus* (Kim et al., 2014), barramundi *Lates*

calcarifer (Siddik et al., 2018b), and Snubnose pompano *Trachinotus blochii* (Pham et al., 2022). Another hydrolysate from shrimp, termed shrimp hydrolysate (SH), prepared by enzymatic hydrolysis of wastes from the shrimp processing industry contains protein, lipid, carotenoid and chitin (Gildberg & Stenberg, 2001). SH also contains bioactive peptides which may perform as growth stimulating agents in aquatic feeds (Gildberg & Stenberg, 2001). The positive effect of dietary inclusion of SH on growth was recorded in red seabream *Pagrus major* (Bui et al., 2014).

Meat and bone meal (MBM) is also one of the promising alternatives to fishmeal protein sources; it has been popularly used in poultry feed (Ai et al., 2006) due to its high protein levels and more beneficial effects on the growth performance of poultry (Ai et al., 2006). In aquaculture, MBM has been reported to partially replace FM without compromising the growth and feed utilization in spotted rose snapper *Lutjanus guttatus* fed 252 g/kg MBM (Hernández et al., 2016), Florida pompano fed 100 g/kg MBM (Rossi Jr & Davis, 2014), Korean rockfish *Sebastes schlegeli* fed 123 g/kg MBM (Yan et al., 2014), rainbow trout *Oncorhynchus mykiss* fed 120 g/kg MBM (Bureau et al., 2000). However, reduced growth performance was observed in Japanese amberjack *Seriola quinqueradiata* fed 192 g/kg MBM, large yellow croaker *Pseudosciaena crocea* fed more than 325 g/kg MBM (Ai et al., 2006) and turbot fed 342 g/kg MBM (Song et al., 2016). Lower digestibility and imbalanced essential amino acids may be attributed to the adverse responses in fish fed high MBM - based diets (Ai et al., 2006). Therefore, there is a need to investigate ingredient supplementation to improve digestibility and overall desirable characteristics of the diet, including MBM as a partial replacement of fishmeal protein.

FPH may increase the efficacy of alternative to fishmeal protein in aqua-diets as its positive effect on the growth performance of barramundi (Siddik et al., 2019) and Snubnose pompano (Pham et al., 2021b) fed high poultry by-product meal diets are established. FPH also improves the growth and protein retention of oliver flounder (Zheng et al., 2014) and turbot (Xu et al., 2017) fed high plant-based diets. Further, FPH inclusion improves the growth and innate immunity of European seabass *Dicentrarchus labrax* (Costa et al., 2020), and restores the intestinal inflammation of Florida pompano (Novriadi et al., 2017) fed plant-based diets. Supplementation of

FPH may provide essential amino acids which are usually limited in alternative protein sources, thereby improving the quality of these diets (Siddik et al., 2019). FPH also acts as an attractant and thus enhances the palatability of feed (Chotikachinda et al., 2013). High digestibility of FPH due to the availability of low molecular weight peptides and free amino acids (Ospina-Salazar et al., 2016; Refstie et al., 2004; Siddik et al., 2018b), is also a documented finding (Kader et al., 2012; Khosravi et al., 2015c). Furthermore, bioactive properties in FPH stimulate immune function and disease resistance, as reported in red sea bream (Khosravi et al., 2015a), Japanese seabass *Lateolabrax japonicus* (Liang et al., 2006), coho salmo *Oncorhynchus kisutch* (Murray et al., 2003).

Studies on the requirements of protein and lipid with corresponding P/E ratio, and the use of FPH or MBM as partially alternative protein sources have been reported in several marine fish but are still novel in juvenile giant trevally. Therefore, this research investigated protein and lipid requirements of giant trevally, and evaluated if different FPH sources at several dietary inclusions could have any beneficial effects while simultaneously replacing a part of dietary fishmeal. Furthermore, the research investigated if FPH could improve the efficacy of an MBM diet in juvenile giant trevally.

1.2. Aim

The overall aim of the study is to evaluate dietary protein and lipid requirements with corresponding P/E ratios in a juvenile giant trevally and investigate the effects of various dietary hydrolysates as a fishmeal supplementation on the growth, feed utilisation, body composition, and physiological and haematological response of juvenile giant trevally fed fishmeal and high MBM-based protein diets.

1.3. Objectives

The aim of the research is achieved by meeting the following objectives:

a. To evaluate the growth, feed utilisation and body composition of juvenile giant trevally fed diets with different levels of P/E ratios.

b. To evaluate the growth, feed utilisation, body composition and physiological responses of juvenile giant trevally when dietary fishmeal protein is substituted by various levels of fish protein hydrolysates.

c. To investigate the growth, physiological and heamatological responses of juvenile giant trevally fed tuna hydrolysates and shrimp hydrolysate as partial alternatives to fishmeal protein.

d. To evaluate whether dietary FPH supplementation can improve the efficacy of a MBM diet in juvenile giant trevally.

1.4. Significance

The specific significance of the research is detailed below:

- The research will contribute to understanding the importance of suitable protein to energy ratio in the formulated diet of juvenile giant trevally.
- The research will contribute to the basic knowledge of nutritional requirements of juvenile trevally.
- The research will highlight the beneficial effects, if any, of supplementing hydrolysates in the diets of the juvenile giant trevally.
- The research will reduce the reliance on fishmeal in the diets of juvenile marine finfish species by finding alternative protein sources in the form of meat and bone meal.
- The research will evaluate if dietary hydrolysate sources can be effective for improving growth, feed utilisation and physiological responses in juvenile trevally.
- The research will assess if hydrolysate improves the efficacy of a meat and bone meal diet in giant trevally.
- Finally, the research outcome will advance towards developing a sustainable formulated diet for the giant trevally.

CHAPTER 2. LITERATURE REVIEW

2.1. General global aquaculture

During recent decades, aquaculture has rapidly developed and importantly contributed to global food security for humans. Annual global aquaculture production was only 43.4 million tons in the 2000s. It rapidly increased to 87.5 million tons in 2020 (FAO, 2022). Inland aquaculture accounted for a higher volume of 62.2% with 54.4 million tons and the remaining production was sourced from marine aquaculture (Table 1). Asia dominated aquatic animal aquaculture with 88.5% in 2020, with major producers including China, India, Indonesia, Vietnam, and Bangladesh (FAO, 2022). The second and third positions belonged to the Americas (5.0%) and Europe (3,8%), with their top representatives being Chile and Norway, respectively. Regarding species groups, finfish contributed 57.5 million tons, accounting for 65.7% of global aquaculture in 2020, followed by molluscs and crustaceans with 17.7 million tons and 11.2 million tons, respectively (Table 2).

Table 1. Global aquaculture production (million tons).

Aquaculture	2000s	2010s	2018	2019	2020
	Average per year				
Inland	25.6	44.7	51.6	53.3	54.4
Marine	17.9	26.8	30.9	31.9	33.1
Total	43.4	71.5	82.5	85.2	87.5

Sourced from FAO (2022)

Table 2. Aquaculture production by region and species groups in 2020 (million tons).

Species group	Asia	Americas	Europe	Africa	Oceania	World
Finfish	50.0	2.4	2.7	2.2	0.1	57.5
Crustaceans	9.9	1.4	-	-	-	11.2
Molluscs	16.4	0.7	0.6	-	0.1	17.7

Other aquatic animals	1.1	-	-	-	-	1.1
Total	77.4	4.4	3.3	2.3	0.2	87.5

Sourced from FAO (2022)

2.1.1. Aquaculture of marine finfish

Marine finfish aquaculture has been practiced in sea-cages inshore and offshore or ponds onshore. This culture had only taken place on grow-out sites, which initially depended on wild seeds. In recent years, the success of marine hatchery, the development of grow-out technologies and the improvement of global trade finfish have boosted the development of marine finfish aquaculture with significantly increased production and abundance of high-valued species. Its production significantly increased from 1839.6 thousand tons in 2000 to 6090.8 thousand tons in 2020 for some major marine finfish species (FAO, 2022) (Table 3). Wherein, Atlantic salmon *Salmo salar* and milkfish *Chanos chanos* contributed the highest volume to the mariculture production. Marine finfish aquaculture has been moved from inshore with small simple culture systems to offshore in deep and open ocean waters with highly advanced technological systems of large volume HDPE cages in some developed countries (Naylor et al., 2000). For example, Norway and China are the leaders in offshore marine aquaculture with the use of large, submerged cages (Thomas et al., 2019). However, aquaculture offshore faces many challenges, including high investment and operating costs, and high fluctuation of environmental parameters, which need many novel design approaches (Naylor et al., 2000).

Table 3. Aquaculture production of major marine finfish species (thousand tons) (FAO, 2022).

Species	2000	2005	2010	2015	2020
Atlantic salmon, *Salmo salar*	895.7	1266.6	1433.8	2380.2	2719.6
Milkfish, *Chanos chanos*	429.7	542.9	750.5	1012.3	1167.8

Mullets nei, *Mugilidae*	92.4	173.7	102.7	129.2	291.2
Gilthead seabream, *Sparus aurata*	87.3	110.8	142.3	168.8	282.1
Large yellow croaker, *Larimichthys croceus*	0	90.9	83.3	142.4	254.1
European seabass, *Dicentrarchus labrax*	60.7	60.9	118.0	149.1	243.9
Groupers nei, *Epinephelus* spp.	7.6	57.1	77.2	149.2	226.2
Coho salmon, *Oncorhynchus kisutch*	108.6	115.1	124.8	140.7	221.8
Japanese seabass, *Lateolabrax japonicus*	0.6	79.6	104.8	120.6	196.9
Pompano, *Trachinotus ovatus*	0	0	80.0	110.0	160.0
Japanese amberjack, *Seriola quinqueradiata*	136.8	159.7	138.9	140.3	137.1
Barramundi, *Lates calcarifer*	18.1	27.0	52.7	68.7	105.8
Red drum, *Sciaenops ocellatus*	2.1	42.4	53.0	71.3	84.3
Total	1839.6	2726.7	3262.0	4782.8	6090.8

2.1.2. The status of marine finfish aquaculture in Vietnam

Vietnam has a long beach of 3260 km with more than 2000 islands, lagoons and bays, where are natural habitats of many aquatic animals. This is a significant advantage for developing marine finfish aquaculture (Tổng cục Thủy sản, 2022). Marine aquaculture was begun in the 1960s with small wood cages located inshore, depending on wild

seeds; its low production only served as food for the farmers' families or was traded in small local markets. In recent years, the success in the hatchery and grow-out techniques of many high valued marine fish species such as cobia, groupers, snapper, barramundi, pompano, milkfish has pushed the development of marine aquaculture in many provinces including Khanh Hoa, Binh Thuan, Quang Ninh, Hai Phong, Vung Tau, and Kien Giang, with different systems such as inshore and offshore sea-cages and pens inland. The production of marine finfish aquaculture has increased from 15,751 tons in 2010 to 58,000 tons in 2021 (Tổng cục Thủy sản, 2022).

However, marine finfish aquaculture in Vietnam has faced some struggles which must be resolved to achieve sustainable development. Although there are high technological systems with large round HDPE cages offshore, most culture systems are simple wood cages that are suitable inshore (Tuổi trẻ, 2023). Besides, feed for fish culture is mostly from trash fish. Specific-species formulated feeds for some fish are still not produced because of inadequate information about nutrition. The gathering of inshore marine finfish aquaculture together with using trash fish for feeding fish lead to a high risk of pollution and disease outbreak. To sustain development of marine finfish aquaculture, the Vietnam government has focused on area planning, technological development of culture offshore and nutritional research (Con số sự kiện, 2020).

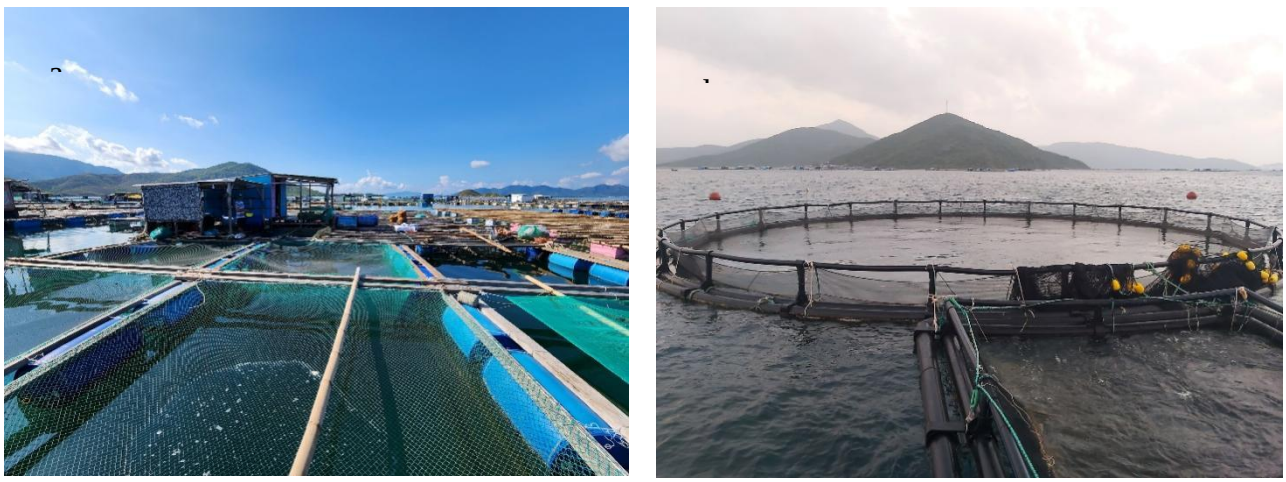


Figure 1. Sea-cage culture of marine finfish in Vietnam. (a) small cages inshore; (b) round large cages offshore.

Figure 2. Trash fish for marine finfish culture in Vietnam.

2.2. Role of nutrition in marine finfish aquaculture

Nutrition plays a crucial role in fish aquaculture, wherein fish require adequate essential nutrients of amino acids from protein, fatty acids from lipids, vitamins, minerals and energy for the physiological demands of their growth (Hixson, 2014). Dietary protein supplies amino acids for protein synthesis in tissue, serving fish growth, while excess protein is catabolized into energy. Dietary lipid is required to supply essential fatty acids and energy, whereas carbohydrate is used as an energy source for fish (Hixson, 2014). In marine finfish aquaculture, protein accounts for the largest proportion ranging from 45% to 55% (Booth et al., 2010; Chou et al., 2001; Glencross, 2006; Nguyen et al., 2018; Suprayudi et al., 2014). It is also the most expensive component in formulated feeds, involving around 60% of the total production cost (Govindharaj et al., 2021). Therefore, feeds for marine finfish need to be formulated with balanced nutrients to satisfy species-specific nutrient requirements and generate cost-effective diets.

2.3. Fishmeal requirements in marine finfish

Fishmeal is made from small fish such as sardine, anchovy, capelin, menhaden, cod and haddock (Boyd, 2015). Materials for annual fishmeal production normally account for one-third of the total global capture fishery. Fishmeal contains high levels of biological protein with excellent amino acid balance for fish species (Barlow, 2003) (Table 4). High essential amino acids, especially lysine and sulfur amino acids, ensure

high nutritional levels in fishmeal diets, and supplement the amino acid deficient in non-fishmeal protein diets (Barlow, 2003). Moreover, fishmeal provides an appreciable lipid content which generates energy for fish activity and contributes to fatty acid synthesis (Masagounder et al., 2016). Fishmeal is a source of minerals such as calcium, phosphorus, sodium chloride, magnesium, iron, zinc and selenium; and cholesterol and certain vitamins, all of which are necessary for the metabolic function of fish (Barlow, 2003; Masagounder et al., 2016). Around 97% of global annual fishmeal production is used in animal feeds, where aquaculture feed accounts for the highest proportion at 69%, followed by pig feed at 23% and poultry feed at 5% (Boyd & McNevin, 2022). The proportion of fishmeal in aquafeed is distributed into major groups as follows: salmonids, 27%; crustaceans, 26%; marine fish, 26%, eels, 5%; cyprinids, 5%; others, 6% (Boyd, 2015). Marine finfish, especially carnivorous fish species, require high protein which is mostly sourced from fishmeal (Boyd, 2015; Saleh et al., 2022). For example, cobia *Rachycentron canadum* requires required 450-500 g/kg protein from fishmeal for optimum growth (Craig et al., 2006; Chou et al., 2001). Similarly, barramundi (Glencross, 2006), permit (Nguyen et al., 2018), blue trevally *Caranx melampygus* (Suprayudi et al., 2014) achieved optimum growth at dietary fishmeal protein levels of 450-550 g/kg, 500 g/kg and 450 g/kg, respectively. Fishmeal requirement for marine finfish aquaculture has been continuously increasing with the rapidly expanding development of this farming sector (Boyd, 2015).

Table 4. The nutritional value of some fishmeal sourced from different fish species.

	White-fish meal	**European herring-type fish meals**	**South American anchovy-type fish meals**	**Menhaden fish meal**
Proximate analysis (%)				
Moisture	10	9	9	8
Crude protein	65	70	65	61
Crude fat	5	9	9	10
Crude ash	20	10	16	19
Crude fiber	0	0	0	0

Sourced from Barlow (2003)

2.4. Drawbacks of using fishmeal in marine aquaculture

Fishmeal is an excellent protein source for fish, but its high demand has put fishmeal sources under the pressure of inadequate supply for aquaculture (Gatlin III et al., 2007). The production of fed fish tripled from 2000 to 2017 (Tacon, 2020), whereas the production of capture fisheries used as raw for fishmeal production decreased from 23 million tons to 16 million tons (Naylor et al., 2021), contributing to the decline of global fishmeal production from 6.6 million tons to 4.8 million tons (FAO, 2020). Increased demand while reduced supply of fishmeal has more than doubled since the 2000s (Naylor et al., 2021). In other words, future fishmeal availability could impede the sustainable development of the aquaculture industry, including marine finfish aquaculture. This has resulted in attempts to substitute fishmeal with other alternative protein sources which contain higher or at least equal nutritional levels compared to fishmeal (Wang et al., 2006).

2.5. Potential alternative protein sources in aqua-feeds

Efforts have been made to produce low-fishmeal diets mainly using plant protein. Soybean meals have been used in the commercial farming of fish and crustaceans as an alternative protein source for fishmeal (Molina-Poveda et al., 2013). Lupin meals are promising protein sources because of their high protein content and balanced amino acid profile (Molina-Poveda et al., 2013). Despite possessing many desirable characteristics as being an excellent alternative protein source for aqua-feeds, plant protein has low digestibility, contains several anti-nutritional factors, and lacks lysine and methionine (Francis et al., 2001; Gatlin III et al., 2007). Additional processing steps have been applied to dismiss anti-nutritional factors and increase the nutritional value of plant protein ingredients (Drew et al., 2007). However, replacing high plant protein levels in diets affects the feed utilisation of carnivorous fish species which find it difficult to digest non-soluble carbohydrates and starch in plant meals (Naylor et al., 2021). It also alters the microbiome and gut morphology, affects the immunology of fish and increases disease risks in fish (Martin & Król, 2017).

In light of these circumstances, alternatives have veered towards other cost-effective and high nutritional sources generated from by-product of animals. Poultry by-product

contains high protein content with a balanced amino acid profile, similar to fishmeal except for lower methionine and lysine (Ayadi et al., 2012). Poultry by-product has been used in commercial diets as partial fishmeal replacement for many fish species such as hybrid striped (*Morone chrysops* × *Morone saxatilis)* (Rawles et al., 2009), and rainbow trout (El-Haroun et al., 2009). Another animal protein is meat and bone meal, which is steadily produced in high amounts in the USA, France, Germany, Spain and Denmark (Ayadi et al., 2012; Cascarosa et al., 2012). This meal contains around 38.5-73.6% crude protein, 2.5-15.5% crude lipid, and 13.0-56.5% ash, being an alternative protein source for aquaculture (Hendriks et al., 2002). MBM was reported to partially replace fishmeal in several fish species such as rainbow trout (Esmaeili et al., 2017), gilthead seabream *Sparus aurata* (Moutinho et al., 2017), ussuri catfish *Pseudobagrus ussuriensis* (Tang et al., 2018), and large yellow croaker (Ai et al., 2006). Although its less than ideal nutritional value together with limited lysine and methionine contents in animal protein does not provide a total fishmeal replacement, this protein source is still an important alternative to fishmeal (Ayadi et al., 2012).

Fishery and aquaculture by-products which are readily available, are potential alternative protein sources in aquafeed (Dekkers et al., 2011; Stevens et al., 2018). Fishmeal produced from by-products contains high nutritional value with 52-67% crude protein, 7-15% crude lipids and 12-23% ash (Jeon et al., 2014; Kim et al., 2014; Ween et al., 2017). Previous studies demonstrated the efficacy of by-product fishmeal as an alternative protein source in aquafeed. Kim et al. (2014) found no change in the growth of oliver flounder fed dietary by-product meal replacing 30% fishmeal. Similarly, tuna by-product meals could replace fishmeal in diets for Korean rockfish without affecting growth (Kim et al., 2018). Besides being raw for by-product fishmeal production, fisheries and aquaculture by-products are also processed to fish protein hydrolysate using enzymatic. FPH has high digestibility, well-balance amino acids and especially contains high short-chain peptide and free amino acids, which are easily digested, improving the growth and feed utilization of fish (Ospina-Salazar et al., 2016; Siddik et al., 2018b). FPH was also reported to enhance the immune response and disease resistance of fish (Chaklader et al., 2020a; Tang et al., 2008). Therefore, FPH is used as an attractant, palatability and digestibility enhancer, and

immune stimulant in aquaculture production (Patekar et al., 2023). Having possession of many favorable characteristics, fish by-products and their product, FPH would be future protein materials in aquaculture. The use of fish by-products in aquafeed would result in additional advantages including limiting environmental pollution, caused by by-product discharge, increasing economic and social benefits, and contributing to the sustainable development of aquaculture (Stevens et al., 2018).

2.6. Protein and P/E ratios in aquafeeds

Protein is one of the most crucial components for maintenance, growth, energy supply and reproduction in fish (Cuzon & Guillaume, 1997) and accounts for the largest proportion of feed cost (Govindharaj et al., 2021). Therefore, the establishment of optimum protein levels for growth and reproduction is essential to formulate balanced, cost-effective diets for aquaculture. Dietary protein requirement depends on amino acid profiles and protein to energy ratio in diets (Hixson, 2014). Sufficient energy intake from non-protein sources ensures dietary protein is used for growth (Cuzon & Guillaume, 1997). Excess energy intake results in lipid deposition, reduction of food consumption and consequent effects on the growth of animals (Cuzon & Guillaume, 1997; Ghiasvand et al., 2012). Conversely, less energy from non-protein sources leads to the catabolism of dietary protein to energy rather than muscle protein synthesis (Cuzon & Guillaume, 1997; Saoud et al., 2012). Therefore, a balanced P/E ratio in aqua-feeds is a top priority in fish nutritional studies. Studies on protein to energy ratio have been carried out in many marine carnivorous fish (Table 5). Juvenile permit gained maximum growth when fed diets with a 50% protein and protein to energy ratio of 20.9 g/MJ (Nguyen et al., 2018). A lower dietary protein requirement of 42.5% and CP/CE of 12.8 g/kcal were required for optimal growth of juvenile Asian seabass (Catacutan & Coloso, 1995). Digestible protein level of 35.5-36.5% and DP/DE ratio of 21-22g/MJ resulted in the highest growth of juvenile Florida pompano (Riche, 2009), whereas, kingfish weighing < 200g needed 45.6% digestible protein and 38 g DP/MJ DE for their optimal growth (Booth et al., 2010). Shiau and Lan (1996) recorded a non-significant change in the growth of juvenile Malabar grouper *Epinephelus malabaricus* with a decrease in protein from 50% to 44% while maintaining an energy level of 340 kcal per 100g. The authors posited that protein may

be spared by energy from non-protein sources, allowing more efficient protein utilisation.

Lipids and carbohydrates are energy sources for fish. However, fish's ability to use carbohydrates varies depending on the species and their natural diets. Therefore, lipid is usually used as a major energy source for many carnivorous fish, saving much protein for growth purposes (Bonvini et al., 2015; Hixson, 2014; Regost et al., 2001; Wang et al., 2017). The protein-sparing effect of energy sourced from lipids has been recorded for several fish species (Hung et al., 2017; Kim & Lee, 2005; Ling et al., 2006; Nguyen et al., 2018; Santinha et al., 1999; Wang et al., 2017). Increase in dietary lipids from 6% to 9% could decrease protein requirement for red-spotted grouper *Epinephelus akaara* from 52% to 48% without affecting their growth (Wang et al., 2017). A similar trend was observed when lipids were reduced from 16% to 12% in 55% protein diets of turbot (Liu et al., 2014). In trevally, Suprayudi et al. (2014) observed the protein-spare effect of energy sourced from lipids for growth in juvenile bluefin trevally. The juvenile of this species achieved the maximum growth at dietary protein level of 45% and P/E ratio of 9 kcal/g. However, in diets with the lowest protein level of 33%, the increase in P/E ratio from 9 to 11 kcal/g resulted in improved feed consumption, feed efficiency and protein retention (Suprayudi et al., 2014). Lipids can be efficiently used to spare protein. However, dietary lipid requirements for marine fish have been reported to be low, at <9% for bluefin trevally (Suprayudi et al., 2014), 6-12% for cobia (Craig et al., 2006; Chou et al., 2001), 14-16% for Asian seabass (Glencross, 2006), and 20% for pompano (Nguyen et al., 2018). Excess dietary lipids may reduce the growth performance of fish (Bonvini et al., 2015; Regost et al., 2001; Wang et al., 2017). Thus, determining the optimum protein and lipid requirements with suitable P/E ratios for each species is a crucial step towards the development of nutritionally balanced and cost-effective diets.

Table 5. Protein requirement and P/E ratio for the optimum growth of some fish.

Species	Protein requirement	P:E ratio	Reference
Bluefin trevally *Caranx melampygus*	45%	9 kcal/g	Divakaran et al. (1999)
Barramundi	42.5	12.8 g/kcal	Catacutan and Coloso

Lates calcarifer			(1995)
Barramundi *Lates calcarifer*	45-55%		Glencross (2006)
Permit *Trachinotus falcatus*	50%	20.9 g/MJ	Nguyen et al. (2018)
Kingfish *Seriola lalandi*	45.6%*	38 g/MJ*	Booth et al. (2010)
Florida pompano *Trachinotus carolinus*	35.6-36.6%*	23.8-25.1 g/MJ*	Riche (2009)
Malabar grouper *Epinephelus malabaricus*	44%	3.4 kcal/g	Shiau and Lan (1996)
White sea bream *Diplodus sargus*	38-42%	20 g/MJ	Sa et al. (2006)
Black sea bass *Centropristis striata*	44%	3.5 mg/KJ	Alam et al. (2009a)
Southern flounder *Paralichthys lethostigma*	50%	35.4 mg/KJ	Alam et al. (2009b)
Ussuri catfish *Pseudobagrus ussuriensis*	45%	29.1 mg/KJ	Wang et al. (2013)
Blackspotted croaker *Nibea diacanthus*	47%	24.53 mg/KJ	Li et al. (2017)
Singhi *Heteropneustes fossilis*	40%	98.3 mg/kcal	Khan and Abidi (2012)
Tongue sole *Cynoglossus semilaevis*	55%	110.5 mg/kcal	Liu et al. (2013b)
Hybrid grouper *E. fuscoguttatus x E. lanceolatus*	53.5%	157 mg/kcal	Jiang et al. (2016)
Black sea roach *Rutilus frisii kutum*	42%	19.22 mg/KJ	Ebrahimi et al. (2013)
Turbot *Scophthalmus maximus*	55%	107.1-110.9 mg/kcal	Liu et al. (2014)

Red-spotted grouper *E. akaara*	52% 48%	30.58 mg/KJ 26.88 mg/KJ	Wang et al. (2017)
Chu's croaker *Nibea coibor*	44,4-47,8%		Huang et al. (2017)
Cobia *Rachycentron canadum*	45-50%		Chou et al. (2001); Craig et al. (2006)

** Digestible value*

2.7. Fish hydrolysate protein

2.7.1. Fish hydrolysate protein production

FPH are promising partly alternative protein sources replacing fishmeal in aqua-feed because of their favourite physiological and functional properties (Chalamaiah et al., 2012a; Neklyudov et al., 2000; Siddik et al., 2018b). FPH are prepared by enzymatic hydrolyzing of proteins in the by-products and the discarded waste from the fish processing industry (Chalamaiah et al., 2012b), which are available in huge volumes (Siddik et al., 2021a). Annual seafood production has increased over the years; this level reached 171 million tons in 2016 (FAO, 2018). Fish processing removes fish by-products as waste, which account for more than 60% of fish materials and can cause pollution and disposal problems (Dekkers et al., 2011). These by-products contain relatively high protein materials, which can be used to produce low-market value products such as fishmeal, animal feed and fertilizers (Hsu, 2010). FPH are prepared by enzymatic hydrolyzing of fish proteins in fish waste (Chalamaiah et al., 2012b). The enzymatic hydrolysis converts native proteins in fish waste into smaller peptides containing 2-20 amino acids (Neklyudov et al., 2000).

2.7.2. Nutritional value and biological characteristics of FPH

FPH are highly digestible, have excellent texture, well-balanced amino acids and low molecular weight peptides, which are likely to be easily absorbed to improve biological nutrient uptake (Ospina-Salazar et al., 2016). Short-chain peptides improve feed palatability, resulting in high feed utilization. Moreover, FPH contain high antioxidant peptides, which may protect fatty acid from peroxidation, enhancing the growth and health of the cultured animals (Siddik et al., 2018b). FPH supplementation

in diets has been reported to improve growth performance, nutrient utilization and immune responses of fish (Hevroy et al., 2005; Ospina-Salazar et al., 2016; Refstie et al., 2004; Siddik et al., 2018b; Xu et al., 2016). Also, fish fed dietary FPH have been shown to have enhanced innate immunity and resistance against viral, bacterial and parasitic infections (Bui et al., 2014; Chaklader et al., 2020a; Kotzamanis et al., 2007; Tang et al., 2008).

2.7.3. The role of FPH in fish aquaculture

a. Growth performance

FPH contain short-chain peptides which improve feed palatability and feed utilization, consequently enhancing growth performance (Hevroy et al., 2005; Ospina-Salazar et al., 2016; Refstie et al., 2004; Siddik et al., 2018b; Xu et al., 2016). As shown in Table 6, the moderate FPH levels to replace fishmeal in diets were reported to be beneficial for the growth performance of fish (Bui et al., 2014; Hevroy et al., 2005; Siddik et al., 2018b). Hevroy et al. (2005) claimed that the higher growth of Atlantic salmon was achieved when fed 18-24% dietary fishmeal substituted by FPH, corresponding to 91-124 g kg^{-1} FPH inclusion levels. Similarly, improved growth was observed at moderate FPH inclusion levels of 100 g kg^{-1} in European sea bass (Kotzamanis et al., 2007) and 51.2 g kg^{-1} and 154.3 g kg^{-1} in Atlantic salmon (Refstie et al., 2004) whereas low FPH levels of 30.5-61 g kg^{-1} in exchange of 5-11% fishmeal enhanced the growth performance of Asian seabass (Siddik et al., 2018b), red seabream (Bui et al., 2014), and Japanese flounder (Zheng et al., 2014). However, high dietary FPH levels caused reduced growth performance in turbot (Xu et al., 2016), Japanese flounder (Zheng et al., 2014), Asian seabass (Siddik et al., 2018a), pompano (Pham et al., 2022). Kim et al. (2014) reported that more than 280 g kg^{-1} FPH resulted in poor growth and feed utilization in olive flounder. Reduced growth and feed utilization were also observed in Japanese flounder fed a diet containing 67 g kg^{-1} FPH (Zheng et al., 2014), whereas this threshold level in turbot was 124 g kg^{-1} (Xu et al., 2016). Especially, deleterious effects on growth, feed utilization, and digestibility were observed in juvenile Asian seabass fed diets containing 415 and 5819.5 g kg^{-1} tuna hydrolysate (Siddik et al., 2018a). The excessive amount of low-molecular-weight peptides and free amino acid in FPH can lead to saturation in the peptide transport mechanism (Ospina-Salazar et

al., 2016) and the amino acid imbalance in fish gut (Kolkovski & Tandler, 2000). Short peptides in FPH go through the intestine wall too fast, possibly causing the saturation of the digestible system (Ospina-Salazar et al., 2016). Free amino acids in FPH can be absorbed quickly, but the fast absorbance of a large amount of free amino acid might contribute to amino acid imbalance (Kolkovski & Tandler, 2000). Moreover, short-chain peptides can improve the feed palatability but create a bitter taste; thus, high dietary FPH levels result in decreased palatability and increased diet bitterness, lowering feed utilization and growth performance (Siddik et al., 2018b).

Table 6. Effects of FPH on the growth, feed utilization, body composition and physiological response of some fish.

Species	Dose FPH (g kg^{-1})	FM substituted by FPH (%)	Response	Reference
Atlantic salmon	30	6	↑ Growth at 92 and 121 g kg^{-1}	Hevroy et al. (2005)
Salmo salar	61	12	↔ Body composition	
	92	18	↔ Hematological parameters	
	121	24		
	150	30		
Atlantic salmon	51.2	-	↑ Growth, PER at 51.2 and 154.3 g kg^{-1}	Refstie et al. (2004)
Salmo salar	102.8	-	↓ PER at 102.8 g kg^{-1}	
	154.3	-	↔ Whole-body protein	
Large yellow croaker	50	-	↑ Growth at 100 and 150 g kg^{-1}	Tang et al. (2008)
Pseudosciaena crocea	100	-	↑ Lysozyme, serum complement, IgM at 100 and 150 g kg^{-1}	
	150	-		
Persian sturgeon	44.9	10	↑ Growth at 44.9 and 187.3 g kg^{-1}	Ovissipour et al. (2014)
Acipenser persicus	187.3	25	↑ Whole-body protein at 44.9 and 187.3 g kg^{-1}	
	374.7	50	↓ Whole-body protein at 374.7 g kg^{-1}	
Pike silverside	81.8	15	↓ Growth at 164 and 245 g kg^{-1}	Ospina-Salazar et al. (2016)
Chirostoma estor	164	30		
	245	45		

Japanese flounder	35	5	↓ Growth at 280, 420, 560 and 700 g kg^{-1}	Kim et al. (2014)
Paralichthys olivaceus	70	10		
	140	20		
	210	30		
	280	40		
	420	60		
	560	80		
	700	100		
Baramundi	415	50	↓Growth	Siddik et al. (2018a)
Lates calcarifer	589.5	75	↔ VSI, HSI, CF and survival	
			↔ Body protein, lipid, ash	
			↔ Hematological parameters	
			Damage liver at 589.5 g kg^{-1}	
Japansese seabass	27	5	↑ Growth at 81 g kg^{-1}	Liang et al. (2006)
Lateolabrax japonicus	81	15		
	136	25		
Japanese flounder	23	6	↑ Growth and protein retention at 45 g kg^{-1}	Zheng et al. (2014)
Paralichthys olivaceus	45	11	↑ Body protein and lipid at 23 and 45 g kg^{-1}, then ↓ at 67, 90 and 110 g kg^{-1}	
	67	16		
	90	21	↑ IGF-I mRNA at 45 g kg^{-1} compared to 110 g kg^{-1}	
	110	26		

Japanese flounder	12	-	↑ Growth, body protein at 37 g kg^{-1} untrafiltration FPH	Zheng et al. (2012)
Paralichthys olivaceus	37	-	↑ Protein retention efficiency at 37 g kg^{-1} untrafiltration and non-untrafiltration FPH	
			↑ Plasma IGF-I level at 12 and 37 g kg^{-1} FPH	
			↑ Liver IGF-I m RNA level at 37 g kg^{-1} untrafiltration FPH	
Red seabream	50	10	↑ Growth and feed utilization at 50 g kg^{-1} krill hydrolysate and shrimp hydrolysate	Bui et al. (2014)
Pagus major			↔ Body composition	
			↔ Hematological response	
Japanese flounder	44	10	↑ Growth and feed utilization at 44 g kg^{-1} krill hydrolysate	Khosravi et al. (2015a)
Paralichthys olivaceus			↑ Dry matter digestibility at 44 g kg^{-1} krill hydrolysate and tuna hydrolysate	
			↑ Dry serum total protein at 44 g kg^{-1} krill hydrolysate and shrimp hydrolysate	
			↔ Body composition	
European seabass	100	-	↑ Growth, survival, intestine development at 100 g kg^{-1}	Kotzamanis et al. (2007)
Dicentrarchus labrax	190	-		
Turbot	31	5	↓ Growth, feed utilization at 124 g kg^{-1}	Xu et al. (2016)
Scophthalmus maxim	62	10	↓ Body protein, lipid at 124 g kg^{-1}	
	124	20	↑ Body ash at 124 g kg^{-1}	
Barramundi	30.5	5	↑ Growth at 30.5 and 61 g kg^{-1}	Siddik et al. (2018b)
Lates calcarifer	61	10	↓ Blood glucose at 61, 91.5 and 122 g kg^{-1}	

	91.5	15		
	122	20		
Snubnose pompano	30	5	↑ Growth at 60 g kg^{-1}	Pham et al. (2022)
Trachinotus blochii	60	10	↔ Feed utilization, somatic indices, body composition	
	90	15	↓ Serum total protein at 120 g kg^{-1}	
	120	20		

Increase ↑, decrease ↓, no change ↔ compared to the control ($P<0.05$).

b. Feed utilization and body composition

Shorter peptides in FPH may perform as attractants to improve palatability and increase feed utilization (Hevroy et al., 2005; Refstie et al., 2004; Siddik et al., 2018b; Xu et al., 2016). Palatability was considered as a reason for the improvement of feed intake of fish fed dietary FPH (Hevroy et al., 2005; Refstie et al., 2004). Higher feed consumption was observed in Atlantic salmon fed 10% and 15% FPH than 5% and 0% FPH (Refstie et al., 2004). Similarly, Hevroy et al. (2005) found that 6% FPH resulted in increased feed intake. However, FPH did not significantly affect the feed intake of Snubnose pompano (Pham et al., 2022), Atlantic cod *Gadus morhua* (Aksnes et al., 2006a), rainbow trout (Aksnes et al., 2006b) and red seabream (Bui et al., 2014), demonstrating that their growth was not the result of palatability. Growth improvement due to FPH supplement might be explained by fast assimilation and effective absorbance of low-weight-molecular peptides and free acids in FPH through the intestine membrane (Aksnes et al., 2006a).

Whole-body lipids in turbot were modulated by dietary FPH inclusion, where lipid content reduced with increasing FPH level (Xu et al., 2016). The concentrations of serum triacylglycerol and cholesterol were decreased by FPH supplementation, proving the lipid-reducing effects of dietary FPH (Xu et al., 2016). Furthermore, whole-body lipids increased and then reduced with increasing FPH in Atlantic salmon (Espe et al., 2012) and Japanese flounder (Zheng et al., 2014), indicating that whole-lipid content reduced at certain levels of dietary FPH. Dietary FPH might decrease the synthesis of fatty acids and stimulate lipid oxidation and energy expenditure, reducing lipid content in the whole-body (Bjørndal et al., 2013; Xu et al., 2016).

Some studies reported no effect of dietary FPH on whole-body protein and ash in red seabream (Bui et al., 2014), turbot (Oliva-Teles et al., 1999), Atlantic salmon (Refstie et al., 2004), barramundi (Siddik et al., 2018a), and Snubnose pompano (Pham et al., 2022). However, whole-body protein of Japanese flounder increased first at 30-45 dietary FPH g kg^{-1} and then decreased with further increasing dietary FPH of 90-110 g kg^{-1} (Zheng et al., 2014). In Persian sturgeon *Acipenser persicus*, whole-body protein increased at 187.3 g kg^{-1} FPH and decreased at 374.7 g kg^{-1} FPH (Ovissipour et al., 2014). Turbot achieved higher whole-body protein in 31-62 g kg^{-1} FPH than higher

FPH levels (Xu et al., 2016). The fast absorbance through the gut wall of a high amount of free amino acids in excessive FPH levels might be responsible for amino acid imbalance (Kolkovski & Tandler, 2000). The imbalance of amino acids probably leads to the degradation of amino acids, consequently reducing protein synthesis in the body of fish (Ovissipour et al., 2014).

c. Physiological responses

Hematological parameters are regarded as the indicators evaluating the physiological stress response and the general health of fish (Rey Vázquez & Guerrero, 2007). Bui et al. (2014) found no change in blood parameters between fish fed diets supplemented and non-supplemented with FPH, demonstrating that nutritional and environmental conditional and tested FPH levels had no adverse effects on the health of red seabream. A similar result was seen in Atlantic salmon, wherein FPH substitution for fishmeal did not impact the health of Atlantic salmon, evaluated by no difference in hematological indices (Hevroy et al., 2005). In contrast, reduced serum total protein was observed in Snubnose pompano fed 120 g kg^{-1} FPH (Pham et al., 2022), and olive flounder fed 44 g kg^{-1} FPH (Khosravi et al., 2015a); while reduced blood glucose was recorded in sea bass fed 61, 91.5 and 122 g kg^{-1} (Siddik et al., 2018b). The inconsistent results in those studies might be caused by different species, FPH inclusion level, fish size, handling method and experimental conditions which strongly impact fish physiology (Pham et al., 2022).

FPH supplementation affected insulin-like growth factor I (IGF-I) in fish (Zheng et al., 2012; Zheng et al., 2014). IGF-I and GH play a central role in the endocrine regulators that mediate the growth and metabolism of vertebrates (Aksnes et al., 2006b). IGF-I and GH stimulate growth performance; they are significantly affected by nutrition, especially dietary protein (Aksnes et al., 2006b). Fasting or reduced dietary protein/energy ratio contributes to increased GH and decreased IGF-I levels (Aksnes et al., 2006b). Liver IGF-I expression is strongly influenced by dietary protein and significantly correlated with the growth rate in Gilthead bream (Gómez-Requeni et al., 2004). Zheng et al. (2014) reported that liver IGF-I expression level in Japanese flounder increased when fed 45 g kg^{-1} FPH compared to 110 g kg^{-1} FPH, generally showing the same pattern with fish growth. Higher plasma IGF-I level and liver IGF-I

expression were obtained in Japanese flounder fed 37 g kg^{-1} FPH than 12 g kg^{-1} FPH, indicating a positive effect of small molecular weight compounds in FPH on plasma IGF-I level and liver IGF-I mRNA expression (Zheng et al., 2012).

d. Immune response and disease resistance

The effects of FPH on the immune response and disease resistance of fish are presented in Table 7. The process of protein hydrolysis produces bioactive sequence, immune-reactive peptides and anti-bacterial properties, which are potent immunomodulators stimulating the non-specific immune defences against pathogens (Kotzamanis et al., 2007; Siddik et al., 2018b). Lysozyme, complement and phagocytes are important components of the non-specific immunity system of fish (Murray et al., 2003), wherein lysozyme belongs to immune defence against parasites, bacteria and virus (Puangkaew et al., 2004) and phagocytes and complement are in immune surveillance and kill invading pathogens (Gasque, 2004). Immunoglobulin M (IgM) is the main component of the humoral immune system, protecting the animal against many diseases (Bachere, 2000). FPH supplementation significantly affected the immunity of fish, as increased lysozyme, complement and IgM were recorded in larger yellow croaker fed dietary FPH levels of 100 and 150 g kg^{-1} (Tang et al., 2008). Bui et al. (2014) claimed that adding 50 g kg^{-1} krill hydrolysate stimulated non-specific immune response of red seabream as enhanced superoxide dismutase and antiprotease activities as well as plasma IgM, increasing resistance to *Aeromonas tarda.* Increased resistance to *Vibrio Anguillarum* was observed in European seabass fed dietary FPH levels of 100-190 g kg^{-1} (Kotzamanis et al., 2007). Siddik et al. (2018b) found that barramundi fed a diet containing 61 g kg^{-1} FPH modulated the resistance capacity against *Streptococcus iniae*, resulting in higher survival rates. Similarly, olive flounder showed increased resistance to *E. tarda* when fed 44 g kg^{-1} krill hydrolysate and decreased infection rate of *V. harveyi* was achieved in barramundi fed 100 g kg^{-1} FPH (Chaklader et al., 2020a).

Conversely, Atlantic salmon and Atlantic cod fed dietary FPH showed poor survival rates after being challenged with *A. salmonicida* (Gildberg et al., 1995) and *V. anguillarum* (Gildberg & Mikkelsen, 1998), respectively. The survival rate of Atlantic cod fed dietary FPH increased in the first 12 days challenging to *V. anguillarum*, but

was not different from the group fed diet without FPH in the 24 days post-challenge (Gildberg & Mikkelsen, 1998). Liang et al. (2006) stated that lysozyme and complement in the blood of Japanese sea bass increased in group containing 81 g kg^{-1} FPH; and phagocytes enhanced at dietary FPH of 27, 81 and 136 g kg^{-1}. However, there was no recorded difference in survival rate after challenging to *V. anguillarum* for 14 days among fish fed diets with or without FPH supplementation (Liang et al., 2006). Similarly, hydrolysate supplementation increased complement activity and phagocytes but unaffected the resistance to *V. anguillarum* of coho salmon (Murray et al., 2003). Supplementation of 44.9-374.7 g kg^{-1} FPH did not affect resistance to *A. hydrophila* in Persian sturgeon (Ovissipour et al., 2014). Improved immune response but no change in disease resistance might be explained by the too high challenge levels, which overcome the effective resistance range enhanced by FPH supplementation (Liang et al., 2006). A sufficient amount of dietary FPH is critical for the stimulant of immunostimulatory effect (Gildberg & Mikkelsen, 1998). The size of peptides in FPH might affect the immune response of fish, as increased SOD was observed in Atlantic salmon fed FPH of 500 to 3000 Da-peptides (Gildberg & Mikkelsen, 1998). According to Murray et al. (2003), applying concentration and size of FPH, which are immunostimulatory *in vitro*, might be insufficient for *in vivo* trials, thus reducing immune efficiency.

Table 7. The effect of FPH on the immune response and disease resistance of fish.

Species	Dose FPH (g kg^{-1})	FM substituted by FPH (%)	Response	Reference
Atlantic salmon	100		↔ Resistance to *Aeromonas salmonicida*	Gildberg et al. (1995)
Salmo salar				
European seabass	100	10	↑ Resistance to *V. anguillarum* at 100 and 190 g kg^{-1}	Kotzamanis et al. (2007)
Dicentrarchus labrax	190	19		
Japanese seabass	27	5	↑ Lysozyme and complement at 81 g kg^{-1}	Liang et al. (2006)
Lateolabrax japonicus	81	15	↑ Phagocytes at 27; 81 and 136 g kg^{-1}	
	136	25	↔ Resistance to *V. anguillarum*	
Large yellow croaker	50		↑ Lysozyme, serum complement, IgM at 100 and 150 g kg^{-1}	Tang et al. (2008)
Pseudosciaena crocea	100			
	150			
Coho salmon	303	-	↑ Hematocrit, complement, phagocytes	Murray et al. (2003)
Oncorhynchus kisutch			↔ Resistance to *V. anguillarum*	
Persian sturgeon	44.9	10	↔ Resistance to *A. hydrophila*	Ovissipour et al. (2014)
Acipenser persicus	187.3	25		
	374.7	50		
Red seabream	50	10	↑ IgM at 50 g kg^{-1}	Bui et al. (2014)
Pagus major			↔ Lysozyme	

			↑ SOD and antiprotease at 50 g kg^{-1} krill hydrolysate ↑ Resistance to *Edwardsiella tarda at 50* g kg^{-1} krill hydrolysate and tuna hydrolysate.	
Olive flounder *Paralichthys olivaceus*	44	10	↑ Lysozyme, MPO, antiprotease at 44 g kg^{-1} krill hydrolysate ↑ Resistance to *E. tarda*	Khosravi et al. (2015a)
Barramundi *Lates calcarifer*	30.5 61 91.5 122	5 10 15 20	↔ Lysozyme and complement ↑ Resistance to *Streptococcus iniae* at 61 g kg^{-1}	Siddik et al. (2018b)
Barramundi *Lates calcarifer*	100	-	↓ Infection rate of *V. harveui* at 100 g kg^{-1}	Chaklader et al. (2020a)
Snubnose pompano *Trachinotus blochii*	30 60 90 120	5 10 15 20	↑ Resistance to *S. iniae* at 60 g kg^{-1}	Pham et al. (2022)

Increase ↑, decrease ↓, no change ↔ compared to the control ($P<0.05$).

e. Supplemented nutrition on high alternative protein diets

The documented findings stated that FPH supplementation improved the efficacy of alternative protein in diets for fish. Atlantic salmon fed 80% plant protein diet supplemented with 10% FPH grew as well as fish fed a control diet containing 35% fishmeal (Egerton et al., 2020). No change in growth, feed utilisation, and immunological and haematological responses was recorded between European seabass fed a plant protein based diet added 3% FPH, and fish fed fishmeal based diet (Costa et al., 2020). Siddik et al. (2021b) reported that supplementation of 46 g/kg FPH allowed the maximum poultry by-product inclusion to replace up to 75% fishmeal without compromising the growth in barramundi. Similarly, positive effects of FPH supplementation on growth, feed efficiency and nutrient retention were noticed in Japanese flounder (Zheng et al., 2014), turbot (Xu et al., 2017) and red sea bream (Khosravi et al., 2015c) fed high plant-based diets and barramundi (Chaklader et al., 2020a) fed high poultry by-product diets. Alternative protein sources contain high protein levels. However their high content in diets is unfavourable for carnivorous fish (Zheng et al., 2014) because they have normally low digestibility, and limited essential amino acids such as methionine and lysine (Shapawi et al., 2007), especially as they contain antinutritional factors as growth inhibitors in plant protein meals (Egerton et al., 2020). Inferior growth and feed efficiency when feeding high levels of plant protein or poultry by-product meals were reported in several fish such as rainbow trout (Gomes et al., 1995), salmon (Refstie et al., 2001), cobia (Zhou et al., 2011), Florida pompano (Rossi & Davis, 2012) (Table 8). FPH are characterized by high contents of short peptides and free amino acids which are easily digested; therefore the presence of FPH may improve digestibility in high alternative protein diets, as reported in red sea bream (Khosravi et al., 2015c). Bioactive properties in FPH seem to be essential promoters of fish growth (Aksnes et al., 2006a). Moreover, FPH may provide supplemented nutrients and energy in high alternative protein diets for the normal growth of fish (Khosravi et al., 2015c).

Table 8. The alternative protein meal inclusion with FPH supplementation for the optimum growth of fish.

Species	Alternative protein source	FPH inclusion level in diet	Reference
Red sea bream *Pagrus major*	Plant protein replaced 50% fishmeal	SH (33,4 g/kg) TH (28,8 g/kg) KH (31,2 g/kg)	Khosravi et al. (2015c)
European seabass *Dicentrarchus labrax*	Plant protein-based diet	FPH (3%)	Costa et al. (2020)
Atlantic salmon *Salmo salar*	80% plant protein diet	FPH (10%)	Egerton et al. (2020)
Olive flounder *Paralichthys olivaceus*	Plant protein replaced 50% fishmeal	SH (33.4 g/kg) TH (28.8 g/kg) KH (31.2 g/kg)	Khosravi et al. (2018)
Barramundi *Lates calcarifer*	90% bioprocessed poultry by-product diet	TH (61 g/kg)	Siddik et al. (2019)
Barramundi *Lates calcarifer*	Poultry by-product replaced 75% fishmeal	FPH (46 g/kg)	Siddik et al. (2021b)
Barramundi *Lates calcarifer*	90% poultry by-product diet.	KH (95 g/kg) CH (9 g/kg) TH (11.8 g/kg)	Chaklader et al. (2020a)
Barramundi *Lates calcarifer*	90% poultry by-product diet	TH (5%) + HI (5%)	Chaklader et al. (2021)

TH: tuna hydrolysate, KH: krill hydrolysate, CH: carp hydrolysate, SH: shrimp hydrolysate, FPH: fish protein hydrolysate; HI: *Hermetia illucens*

Besides, FPH supplementation can heal the impaired welfare of fish fed high non-fishmeal diets. The immune response and disease resistance were enhanced in barramundi fed 90% bioprocessed poultry by-product diet supplemented with 61 g/kg FPH (Siddik et al., 2019). FPH supplementation recovered the innate immunity of red sea bream, which was negatively affected by replacing 50% of fishmeal with plant

meal (Khosravi et al., 2015a). Costa et al. (2020) reported that 3% of FPH could reduce the adverse effects on the immune response of European seabass fed high plant diets and increased bactericidal and bacteriostatic activities against various *Vibrio* species. Similarly, plant protein meal substitution to 75% of fishmeal with FPH supplementation resulted in an increase in white blood cells and a reduction of mortality of barramundi after a 14-day challenge with *Vibrio harvei* (Siddik et al., 2021b). European sea bream fed a plant protein based diet supplemented with 5% shrimp hydrolysate or a mixture of 5% hydrolysate and 5% tilapia hydrolysate showed enhanced immune-related genes and metabolic pathways (Leduc et al., 2018). The improved immune response may be associated with the positive effects on disease resistance against bacteria of the small sized-bioactive peptides in FPH (Siddik et al., 2021b).

2.8. Meat and bone protein production and its use in aquaculture

2.8.1. MBM production and its nutritional values

MBM is a product of rendered animal process; it is one of the promising alternative protein sources with a steady availability and massive production of around 3.5 million tons per year in the EU (Coutand et al., 2008). MBM contains high protein levels and is an excellent source of trace elements, especially phosphorous and calcium; it lacks antinutritional factors (Coutand et al., 2008; Moutinho et al., 2017), and its price is relative lower than fishmeal (Esmaeili et al., 2017). Analysis of *in vitro* digestibility data of the chemical composition of 94 MBM samples in New Zealand showed that the mean protein, lipid and gross energy contents were 56.8%, 10% and 17.1%, respectively, however with huge variable ranges (Hendriks et al., 2002) (Table 9). The quality of MBM was highly variable, mainly depending on raw sources (Johnson & Parsons, 1997) and process techniques for its production (Kureshy et al., 2000).

Nonetheless, the high ash content in MBM, due to the high level of bone and inorganic matter, reduces its digestibility and nutrients' bioavailability, thereby limiting its use in aqua-feed (Robaina et al., 1997). Furthermore, the imbalance of essential amino acids may be also a drawback and cause unexpected responses in fish fed high MBM diets (Ai et al., 2006). Amino acid deficiency in MBM, normally lysine and methionine,

may affect protein synthesis in fish, in turn reducing fish growth. Table 10 shows lower values of most of essential amino acids in MBM than fishmeal. Thermal treatment in the process of MBM production may damage the structure of protein and amino acids, leading to some amino acid efficiency (Wang et al., 2020).

Table 9. *In vitro* digestibility data of chemical composition of 94 meat and bone meal samples in New Zealand.

Component	Range	Mean
Dry matter (%)	91.2-98.6	95.4
Crude protein (%)	38.5-73.6	56.8
Lipid (%)	2.5-18.5	10.0
Ash (%)	13.0-56.5	28.4
Gross energy (kJ/g)	9.4-22.3	17.1

Data was derived from (Hendriks et al., 2002).

Table 10. The essential amino acid of meat and bone meal and fishmeal.

Component	Meat and bone meal	Fishmeal
Arginine (%)	4.2	5.8
Histidine (%)	1.1	2.4
Isoleucine (%)	1.6	4.3
Leucine (%)	3.5	7.2
Lysine (%)	3.0	7.5
Methionine (%)	0.9	2.8
Phenylalanine (%)	1.9	3.9
Threonine (%)	2.0	4.2
Valine (%)	2.4	n/r
Cysteine (%)	0.4	0.8
Tyrosine (%)	1.3	3.0

Data was derived from Hendriks et al. (2002), Petterson et al. (1997) n/r: not reported.

2.8.2. The role of MBM in aquaculture

Several studies have been conducted to investigate the potential use of MBM to replace fishmeal in aqua-feed for various fish such as gilthead seabream (Moutinho et al., 2017; Robaina et al., 1997), sutchi catfish *Pangasius hypophthalmus* (Abdul Kader et al., 2011), large yellow croaker (Ai et al., 2006), ussuri catfish (Tang et al., 2018), rainbow trout (Esmaeili et al., 2017), Korean rockfish (Yan et al., 2014), **s**potted rose snapper (Hernández et al., 2016), olive flounder (Lee et al., 2012), turbot (Song et al., 2016), ussuri catfish (Wang et al., 2020)**,** and gibel carp *Carassius auratus gibelio* (Zhang et al., 2006). Previous studies generally reported that MBM could partially replace dietary fishmeal. However high dietary MBM inclusion levels could compromise growth and feed utilization and even negatively affect the physiological and immunological responses of the host fish. The use of MBM as an alternative protein source for fishmeal replacement is overviewed in Table 11.

a. Growth performance and feed utilization

Studies on fish reported varied dietary MBM levels in exchange of 10-67% fishmeal without growth impairment, such as a replacement of up to 67% in sutchi catfish (Abdul Kader et al., 2011), 50% in gilthead seabream (Moutinho et al., 2017), 15% in Korean rockfish (Yan et al., 2014), and 10% in oliver flounder (Lee et al., 2012) (Table 11). However, high dietary MBM levels depressed fish growth and feed utilization, especially when MBM was used as a sole protein source in diets (Ai et al., 2006). A replacement of 60% FM with MBM significantly reduced the growth of large yellow croaker (Ai et al., 2006), and ussuri catfish (Tang et al., 2018). A substitution level of 45% inhibited the growth and feed efficiency rate of turbot (Song et al., 2016). The reduced growth of fish fed dietary MBM is attributed to poor palatability, low digestibility, and unbalanced fatty acid and amino acid profiles. Studies on large yellow croaker (Ai et al., 2006), rainbow trout (Bureau et al., 2000), and ussuri catfish (Tang et al., 2018) found reduced feeding rate with increasing MBM levels due to low palatability. Excess ash, mineral and water insoluble residue in high MBM diets may impact taste, generate the hardness of diets as well as increase intestine transit, leading to inferior feed efficiency (Tang et al., 2018; Wang et al., 2020). Lower apparent digestibility coefficient (ADC) of protein, lipid and energy of MBM than FM were

noticed in large yellow croaker (Ai et al., 2006), as a decreased trend in ADCs of protein and lipid with increasing dietary MBM level in gilthead seabream (Robaina et al., 1997), and ussuri catfish (Tang et al., 2018). High ash content in MBM mainly contributes to suppressed protein digestibility (Ai et al., 2006; Bureau et al., 1999), whereas the high amount of saturated fatty acids in MBM results in low digestibility of energy and lipid (Allan et al., 2000; Robaina et al., 1997), indicating that increased dietary MBM may account for poor digestibility. The deficiency in n-3 HUFA, especially EPA, DHA and ARA in high MBM diets, which are essential for biological structure and function for cell membrane (Sargent et al., 1999), may additionally impact the normal growth of fish (Xu et al., 2010). Furthermore, fish growth may negatively respond to the unbalanced profile of amino acids in dietary MBM, as essential amino acid synthesis in the body decreased in large yellow croaker fed increasing MBM levels in diets (Ai et al., 2006). Methionine, lysine and threonine are generally limiting acids in alternative protein sources (Hu et al., 2013); only one essential amino acid deficiency leads to poor dietary protein utilization (Wilson, 2003). Ai et al. (2006) assumed that the deficiency of lysine, arginine and threonine in diets containing more than 45% fishmeal substitution with MBM boosted amino acid catabolism into energy rather than protein synthesis served for fish growth. Similarly, reduced methionine levels from 9.6 g/kg to 6.6 g/kg with increasing dietary MBM replacement from 20% to 100% may mainly cause suppressed growth and feed utilization of ussuri catfish (Tang et al., 2018).

b. Body composition

Some studies showed no effect of MBM on body composition of fish (Robaina et al., 1997; Yan et al., 2014)**.** However high dietary MBM reduced crude protein and lipid in ussuri catfish (Tang et al., 2018), rainbow trout (Esmaeili et al., 2017), turbot (Song et al., 2016), and large yellow croaker (Ai et al., 2006) (Table 11). It seems to be caused by poor digestible enzyme activity and high amount of saturated fatty acids in dietary MBM which inhibit the utilization and digestion of protein and lipid (Tang et al., 2018). Indeed, low activities of digestive enzymes were observed in ussuri catfish fed MBM replacing up to 40% and more fishmeal (Tang et al., 2018). High saturated fatty acid and low PUFA in high MBM diets impact lipid metabolism in fish (Ai et al.,

2006). On the contrary, rainbow trout fed 45-65% MBM replacement showed declined protein and elevated lipid in the whole fish body (Esmaeili et al., 2017) and MBM substitution levels of 20-40% increased energy retention in gilthead seabream (Robaina et al., 1997). Deficient essential amino acids in high MBM diets and their low absorption may lead to their catabolism into energy and lipid deposition, resulting in the retardation of protein synthesis and fat accumulation in the fish body (Esmaeili et al., 2017).

Dietary MBM causes a negative response from essential amino acids in fish body which is a precursor of protein synthesis, impacting fish growth. The decreased essential amino acids with increasing MBM at more than 45% was seen in large yellow croaker (Ai et al., 2006), and turbot (Song et al., 2016). The reduction of amino acids in fish fed high MBM diets is associated with unbalanced amino acids that contain poor lysine, threonine, leucine and arginine but high isoleucine, histidine, valine and phenylamine levels (Ai et al., 2006). Tang et al. (2018) recorded that methionine decreased from 9.6 to 6.6 g/kg with increasing dietary MBM substituted for fishmeal from 20% to 100%; these levels were lower than 10.3 g/kg methionine in a fishmeal based-diet. Besides, amino acid structures in MBM may be impaired under rendered process (Xavier et al., 2014); such lysine is greatly sensitive to heat and its levels significantly alter among MBM production batches (Parsons et al., 1997). Therefore, it is inferred that essential amino acid deficiency in high MBM diets suppresses the synthesis of essential amino acids in fish body.

Fatty acid composition in fish body is changed to reflect dietary fatty acids in MBM. This is characterized by high SFA and low n-3 HUFA (Millamena & Golez, 2001). Increasing dietary MBM levels increased C18:1n-9/n-3 HUFA but reduced n-3 HUFA in large yellow croaker (Ai et al., 2006). Similarly, Esmaeili et al. (2017) found elevated SFA but declined PUFA and n-3 LC-PUFA (ARA, EPA and DHA) in rainbow trout fed dietary MBM in exchange of exceeding 25% fishmeal. The substitution level of 30% showed higher SFA and mono fatty acid but lower EPA and n-3 HUFA in olive flounder than fish fed fishmeal diet (Kim et al., 2021). However, MUFA in the tissue of rainbow trout fed 65% fishmeal replacement with MBM did not

reflect its increased level in the diet, properly owing to its utilization for energy production (Esmaeili et al., 2017).

Table 11. The effects of dietary MBM on the growth, feed utilization and body composition of some fish.

Species	Dose MBM (g/kg)	FM substituted by MBM (%)	Response	Reference
Gilthead seabream	409	50	↓ growth and ↑ FI at 75%	Moutinho et al. (2017)
Sparus aurata	615	75	↓ whole-body lipid and energy retention at 75%	
			↔ ADC of protein and energy	
			↔ pepsin, trypsin, lipase, chymotrypsin, total alkaline proteases	
Gilthead seabream	140.4	20	↔ growth and body composition	Robaina et al. (1997)
Sparus aurata	210.6	30	↓ FI at 30% and 40%	
	280.9	40	↓ protein ADC at 20%, 30% and 40%	
Sutchi catfish	82	33	↓ growth and ↑ FI at 100%	Abdul Kader et al. (2011)
Pangasius hypophthalmus	164	67	↓ PER and whole-body protein at 100%	
	245	100		
Large yellow croaker	108.6	15	↓ growth, FER, PR at 60% and 75%	Ai et al. (2006)
Pseudosciaena crocea	217.2	30	↓ whole-body lipid at 60% and 75%; ↑ moisture at 75%	
	325.8	45	↔ amino acids	
	434.4	60	↓ n-3 HUFA but ↑ C18:1n-9/n-3 HUFA at 45%, 60% and 75%	
	543.0	75		
Ussuri catfish	138	20	↓ growth at 60%, 80%, 100%	Tang et al. (2018)
Pseudobagrus				

ussuriensis	276	40	↑ FCR and PER at 80%, 100%; ↓ FI at 60%, 80%, 100%	
	414	60	↓ CF at 80%, 100%; ↑ VSI at 80%, 100%	
	552	80	↓ whole-body protein at 60%, 80%, 100%; ↓ lipid at 40%, 60%, 80%, 100%; ↑ ash at 40%, 60%, 80%, 100%; ↑ moisture at 60%, 100%	
	690	100		
			↓ ADC of protein and dry matter at 60%, 80%, 100%; ↓ ADC of lipid at 40%, 60%, 80%, 100%; ↑ ADC of phosphorus at 20%, 40%; ↓ ADC of phosphorus at 60%, 80%, 100%	
			↓ pepsin, lipase and protease in intestine at 60%, 80%, 100%; ↓ amylase in intestine at 100%; ↓ protease in liver at 40%, 60%, 80%, 100%	
Rainbow trout	149.1	25	↔ growth, FCR	Esmaeili et al. (2017)
Oncorhynchus mykiss	266.3	45	↓ PR, LE at 65%	
	381.6	65	↔ CF, VSI, HSI	
			↓ crude protein at 65%; ↓ moisture at 45% and 65%; ↓ ash at 45%; ↔ lipid	
			↑ MUFA and n-6 PUFA at 25%, 45% and 65%; ↓ PUFA at 65%; ↓n-3 PUFA at 25%, 45% and 65%	
			↑ protein digestibility at 25%; ↓ lipid digestibility at 25%, 45%, 65%; ↑ carbohydrate digestibility at 45%, 65%; ↓ energy digestibility at 45%, 65%.	
Korean rockfish	123	15	↔ growth; FCR; ADC of dry matter, protein, energy	Yan et al. (2014)
Sebastes schlegeli			↔ PRE; ERE	
			↔ whole-body composition	

Spotted rose snapper *Lutijnus guttatus*	252	35	↔ growth, FI, SGR, PER ↓ ADC of dry matter, ↔ protein and energy ↔ whole-body protein, ash; ↓ lipid; ↑ moisture	Hernández et al. (2016)
Olive flounder *Paralichthys olivaceus*	60 120	10 20	↑ growth at 10% ↔ feed consumption, FER, PR, CR, HSI ↔ whole-body protein, moisture, ash; ↓ whole-body lipid at 20% ↔ liver protein, moisture; ↓ liver lipid at 20%	Lee et al. (2012)
Olive flounder *Paralichthys olivaceus*	295	30	↔ growth, FER, PER, PR ↔ CF, VSI, HIS ↔ whole-body composition ↓ tryptophan in whole-body; ↔ other amino acids ↑ SFA; ↓ EPA, n-3 HUFA	Kim et al. (2021)
Turbot *Scophthalmus maximus*	34.2	45	↓ growth, FER, PR, FR, ER ↓ whole-body protein and fat ↓ muscle EAA and NEAA	Song et al. (2016)
Ussuri catfish *Pseudobagrus ussuriensis*	84.9*	40	↔ growth, FE, PER ↓ CF; ↑ VSI ↓ whole-body protein; ↔ whole-body lipid, ash, moisture, phosphorus ↔ ADC of protein, lipid and dry matter	Wang et al. (2020)

				↓ pepsin in stomach; ↔ alpha-amylase and lipase in intestine	
Gibel carp		110	20	↓ growth, FE and ERE at 40-100%	Zhang et al. (2006)
Carassius gibelio	*auratus*	220	40	↓ PRE at 60-100%	
		330	60	↔ body composition	
		440	80	↓ protein and phosphorus ADC at 40-100%; ↓ dry matter ADC at 60-100%; ↔ energy ADC	
		550	100		

* MBM in low fishmeal diets

Increase ↑, decrease ↓, no change ↔ compared to the control ($P<0.05$).

FER feed efficiency ratio; FCR feed conversion ratio; PR protein retention; LR lipid retention; FER feed efficiency ratio; PRE protein retention efficiency; ERE energy retention efficiency; CF condition factor; VSI viscera index; HSI hepatosomatic index; ADC apparent digestibility coefficient.

c. Digestibility of nutrients

Apparent digestibility coefficient (ADC) of animal by-product is generally lower than that of fishmeal (Booth et al., 2013; Kureshy et al., 2000; Moutinho et al., 2017). MBM substituting 20% and more fishmeal resulted in declined ADC protein in gilthead seabream (Robaina et al., 1997). Increased dietary MBM level from 20% to 100% caused decreased protein digestibility from 92% to 83% in gibel carp. (Zhang et al., 2006). In ussuri catfish, protein ADC declined with increasing MBM replacement from 60% to 100% (Tang et al., 2018). Poor protein digestibility in MBM is likely to link to high levels of poorly digested ash (Ai et al., 2006). Meat and bone meal contains bone and inorganic matter, which generates high ash content and inhibits its use in aquafeed because the ash impacts the nutrient's utilization (Robaina et al., 1997). Indeed, a negative correlation between dietary ash content and protein digestibility was noticed in previous studies (Bureau et al., 1999; Pongmaneerat & Watanabe, 1991; Robaina et al., 1997). Robaina et al. (1997) suggested that more than 12.5% ash level in diets significantly reduced protein ADC. Digestibility of MBM lipid and energy reduced with increasing MBM replacement level in rainbow trout (Esmaeili et al., 2017) and gilthead seabream (Tang et al., 2018). Lower lipid and energy digestibility in MBM is determined to be related to high saturated fatty acids which account for a major fraction of fatty acid composition in MBM (Robaina et al., 1997). However, some studies show similar digestibility of MBM protein, lipid and energy compared to that of fishmeal, as seen in gilthead seabream (Moutinho et al., 2017), Korean crockfish (Yan et al., 2014), ussuri catfish (Wang et al., 2020), and spotted rose napper (Hernández et al., 2016). It may be attributed to the supplementation of amino acids and favourable quality of MBM ingredients used in the experiments (Esmaeili et al., 2017).

The digestibility of nutrients is mainly based on digestive enzyme activities (Bu et al., 2017) which may be modulated by nutrition input (Bakke et al., 2010). Digestive enzymes, especially protease, lipase and amylase, play a crucial role in the digestive system, affecting feed efficiency and growth performance (Wang et al., 2020). Dietary MBM did not change digestibility enzyme activities in gilthead seabream (Moutinho et al., 2017). Nevertheless, Tang et al. (2018) found declined activities of protease, lipase

and amylase in the intestine in ussuri catfish fed dietary MBM in exchange of 40% and more fishmeal, as lower pepsin was recorded in the stomach of ussuri catfish (Wang et al., 2020). Low digestive enzyme activities may lead to poor nutrient digestibility; however no change in ADC of protein and lipid was observed in gilthead seabream (Tang et al., 2018) and ussuri catfish (Wang et al., 2020) despite low digestive enzyme activities. The decrease in digestive enzymes may be linked to poor enzyme substrate concentration, caused by poor palatability, low feeding rate and high saturated fatty acids in high MBM diets (Ai et al., 2006; Tang et al., 2018).

d. Physiological and immunological responses

Hepatic alteration has been previously reported in gilthead seabream fed dietary MBM, where replacing exceeding 30% fishmeal with MBM produced liver lipid accumulation while nuclei polarization and necrosis were generated in hepatocytes at the substitution level of 40% (Robaina et al., 1997). The negative impact on the liver could be explained by unbalanced fatty acids with high levels of saturated fatty acids in MBM (Robaina et al., 1997). The alteration in fish liver was reported in fish fed high alternative protein diets; such as Japanese seabass fed diets containing high animal protein blend showed hepatic necrosis (Hu et al., 2013), and small lipid droplets occurred in the liver of rainbow trout fed high yellow kernel meal (Glencross et al., 2004) (Table 12).

SOD and CAT are important antioxidant enzymes in the defence mechanism against free radicals; they remove agents generating oxidation and prevent body fatty acids from superoxide reaction, thereby protecting organisms from oxidative damage and increasing the complex immune system (Deng et al., 2015; Hermes-Lima et al., 1998). T-AOC mirrors antioxidative capacity (Tan et al., 2016). LZM is also involved in the defence mechanism reacting to innate immune response due to its positive effect on anticancer and antivirus capacities (Sikkema et al., 1994). MDA is generated from lipid superoxide, and it is an indicator of oxidative stress (Del Rio et al., 2005). Olive flounder fed 295 g/kg MBM substituting 30% fishmeal in high fishmeal diet showed no alteration in SOD and CAT (Kim et al., 2021), as seen in olive flounder fed dietary meat meal (Ha et al., 2021). In contrast, decreased hepatic SOD, CAT, T-AOC, LZM and slight increased MDA were noticed in ussuri catfish fed MBM replacing 40%

fishmeal in low fishmeal diet, indicating that this MBM level reduced antioxidative capacity and immune response (Wang et al., 2020).

IGFs are hormones regulating protein synthesis and body metabolism serving growth (Valente et al., 2012); their activities are linked to nutritional status (Moriyama et al., 2000). Song et al. (2016) reported the replacement of 45% fishmeal with MBM increased mRNA IGF-I, IGF-IR and IGFBP-1 but decreased IGF-II, IGFBP-2, IGFBP-4, IGFBP-5 and IGFBP-6 expression in turbot. IGF activities were previously reported to negatively correlate with IGFBP-1 but positively correlate with IGFBP-2 (Safian et al., 2012), IGFBP-4, IGFBP-5 and IGFBP-6 (Duan & Xu, 2005; Safian et al., 2012). It inferred that 45% fishmeal substitution with MBM may down-regulate the activities of the IGF signal pathway in turbot (Song et al., 2016). Whereas replacing 40% fishmeal with MBM in a low fishmeal diet decreased hepatic IGF-I gene expression in ussuri catfish (Wang et al., 2020). The same trend was observed in ussuri catfish (Luo et al., 2019), European catfish *Silurus glanis* (Kumar et al., 2017), and cobia (Luo et al., 2012) fed high alternative protein levels. Gene expression hepatic IGF-I may be impacted by essential amino acid deficiency and unbalanced amino acid profiles in alternative protein sources (Luo et al., 2019; Wang et al., 2020), as proved by its decreased levels in Atlantic salmon fed low methionine (Espe et al., 2016), cobia fed low lysin (Luo et al., 2012) in high plant protein diets. In ussuri catfish, low amino acid profile and deficiency of lysin, methionine, isoleucine, leucine, threonine and valine may inhibit hepatic IGF-I expression levels (Wang et al., 2020).

Generally, MBM can be used to partially replace fishmeal in aqua-feed. However high MBM levels may cause mixed unexpected responses in fish. Therefore, the selection of supplemented nutritional sources to overcome undesirable characteristics of MBM is useful to increase the capacity of high MBM levels in fish diets. FPH have been previously reported to effectively reduce the negative impact on the growth and health of fish fed high non-fishmeal protein diets such as plant meal (Costa et al., 2020; Gisbert et al., 2018; Khosravi et al., 2018) and poultry by-product meal (Chaklader et al., 2021; Pham et al., 2021b; Siddik et al., 2019). However, the effects of FPH supplementation in fish, involving giant trevally, fed high MBM diets have yet to be verified, thus need to be investigated.

Table 12. The effect of dietary MBM on the physiological and immunological responses of fish.

Species	Dose MBM (g/kg)	FM substituted by MBM (%)	Response	Reference
Gilthead seabream	140.4	20	↑ lipid deposit in liver at 30%, 40%	Robaina et al. (1997)
Sparus aurata	210.6	30	↑ polarization and isolated necrosis at 40%	
	280.9	40		
Spotted rose snapper	252	35	↔ hematocrit, hemoglobin, TP; ↓ Glu	Hernández et al. (2016)
Lutijnus guttatus				
Oliver flounder	60	10	↔ TP, Glu, GOT, GPT, TGC	Lee et al. (2012)
Paralichthys olivaceus	120	20		
Turbot	34.2	45	↑ IGF-I, IGF-IR; ↓ IGF-II	Song et al. (2016)
Scophthalmus maximus			↓ IGFBP-2, IGFBP-4, IGFBP-6; ↑ IGFBP-1	
Ussuri catfish	84.9*	40	↓ hepatic SOD, CAT, T-AOC, LZM; ↔ MDA, AKP	Wang et al. (2020)
Pseudobagrus ussuriensis			↓ IGF-I	
Olive flounder	295	30	↔ AST, ALT, ALP, T-BIL, TP	Kim et al. (2021)
Paralichthys olivaceus			↓ T-CHO, TG, ALB	
			↔ SOD, LZM	

* MBM in low fishmeal-diets

TP total protein; Glu glucose; GOT glutamate oxaloacetate transaminase; GPT glutamate pyruvate transaminase; TGC tryglyceride; SOD superoxide dismutase; CAT catalase; T-ACO total antioxidant capacity; MDA malondialdehyde; LZM serum lysozyme; AKP alkaline phosphate, SOD superoxide dismutase;

2.9. Giant trevally *Caranx ignobilis*

2.9.1. Taxonomy and biology

Giant trevally has following taxanomy (Fishbase, 2015):

Kingdom	Animalia
Phylum	Chordata
Class	Teleostei
Order	Perciformes
Family	Carangidae
Genus	Caranx
Species	*Caranx ignobilis* (Forsskål, 1775)

The trevally *Caranx* spp. is a commercially important aquacultured genus in the family Carangidae. One hundred and forty-six trevally species have been recorded in the world; they are distributed in tropical, subtropical and temperature waters (Firdus et al., 2020). Adults are pelagic in sand and rock areas; they also live singly in clear lagoons and coral reefs (Lieske & Myers, 1994). They are carnivorous and predate mostly on small pelagic fish, some crustaceans and cephalopods (Sley et al., 2009). Giant trevally *Caranx ignobilis* are likely to be the biggest species in this genus, as reflected by its name. Their largest size was reported to reach up to 1.7 m in length and over 60 kg in weight (Kuiter, 2001). They spawn on shallow seaward reefs and offshore oceans (Myers, 1999).

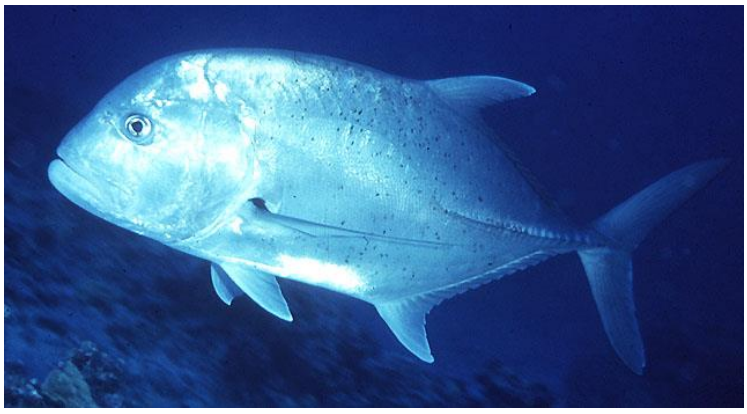


Figure 3: Giant trevally *Caranx ignobilis* (Fishbase, 2015)**.**

2.9.2. Giant trevally aquaculture and nutritional research

a. Aquaculture

Giant trevally have been successfully reproduced in Asia countries (Kappen et al., 2018; Ma et al., 2020). Fish spawned after 24-52 hours 2nd injecting HCG or LHRHa, and eggs hatched between 11-17 hours at 28-30 ppt salinity and 27.6-29.25 ^{o}C (Ma et al., 2020). LHRHa treatment resulted in a higher fertilization rate than HCG treatment, while no difference was observed in mean spawned eggs, hatching rate and larvae number between HCG and LHRHa (Ma et al., 2020). New-hatched larvae are 1.5-1.6 mm in length; they have strong phototropism and experience complete metamorphosis 26-27 days after hatching (Ma et al., 2020). In the nursing stage, fingerling giant trevally showed the highest growth performance and resistance to the temperature at stocking density of 0.5-1.0 fish/L compared to 1.5 and 2.0 fish/L; however no difference was observed in resistance to salinity among stocking densities of 0.5; 1.0; 1.5 and 2.0 fish/L (Pham et al., 2021a).

A study on the grow-out culture of giant trevally was firstly carried out in French Polynesia in 1973-1974. 7-10g-wild seeds were collected and reared in 500 L-tanks with 10 fish/tank; then they were transferred to culture in sea-cages with a stocking density of 900 individual/cage. The result showed that fish reach a mean weight of 420 g after six months with a survival rate of 95% (Aquacop, 1975). Pattipeiluhu et al. (2021) found that giant trevally (14-19 g) reached 180 g after 2.5 months cultured in cages. In Vietnam, giant trevally farming has been most popularly cultivated in Khanh Hoa, Quang Ninh, Vung Tau, Kien Giang due to its high market price, fast growth, comsumer preference and suitability for sea-cage farming (Pham et al., 2021a). Seeds have been produced from the hatchery and collected from the wild (Pham et al., 2021a). This fish has been reared in sea cages and fed with trash fish; they can reach the market size of 800-1000 g after 10-12 cultured months (Pham et al., 2021a).

b. Nutritional research

No published articles are available on the nutritional requirements of *Caranx* spp.; only scant information has been reported for bluefin trevally. Juvenile bluefin trevally require a dietary protein level of 450g/kg and energy to protein ratio of 9 kcal/g for

optimal growth (Suprayudi et al., 2014). The growth and feed consumption were negatively affected when dietary lipid levels exceeded 11% (Suprayudi et al., 2014). Nutritional requirements have been unknown for giant trevally, but reported for several other marine carnivorous fish such as kingfish (Booth et al., 2010), cobia (Craig et al., 2006; Chou et al., 2001), Asian seabass (Glencross, 2006), and pompano. (Lazo et al., 1998; Nguyen et al., 2018). These species require high dietary protein levels of 400-500 g/kg (Booth et al., 2010; Craig et al., 2006; Chou et al., 2001; Glencross, 2006; Lazo et al., 1998; Nguyen et al., 2018; Suprayudi et al., 2014), and a low lipid level of 90-200 g/kg for optimal growth (Craig et al., 2006; Chou et al., 2001; Glencross, 2006; Nguyen et al., 2018; Suprayudi et al., 2014).

2.10. Gaps in nutritional knowledge in aquaculture of giant trevally

Giant trevally is a high-valued cultured species; it has been widely reared in many countries, including Indonesia, Philippine, Malaysia, Vietnam (Kappen et al., 2018; Ma et al., 2020; Pham et al., 2021a). In Vietnam, trash fish is still the main feed for giant trevally, leading to high risks of environmental pollution, pathogen introduction, and unsustainability due to inconsistent supply (Pham et al., 2021a). Species-specific formulated feed for giant trevally is not available owing to the lack of information on its nutritional requirements. Thus, the establishment of balanced formulated feed for giant trevally is essential to achieve sustainable aquaculture potential.

In formulated feed, protein accounts for the largest proportion and highest expensive ingredient (Govindharaj et al., 2021), especially for carnivorous fish as giant trevally. Dietary protein requirement is affected by the presence of energy from non-protein sources in diets; adequate energy leads to converting almost dietary protein into protein in tissue (Hixson, 2014). Thus, a balanced P/E ratio is crucial in diet formulation. Lipids and carbohydrates provide energy for fish. However, carnivorous fish may prefer lipid energy because they find it difficult to digest carbohydrate (Bonvini et al., 2015; Hixson, 2014; Regost et al., 2001; Wang et al., 2017). The determination of dietary protein and lipid requirements with relevant P/E ratio is top priority in the nutritional study for each species. However, this information is limited in giant trevally, and thus needs to be investigated to formulate balanced and cost-effective diets for this species.

Protein in formulated feeds is mainly sourced from fishmeal; however, fishmeal supply has not met the demand because of the rapid development of aquaculture and declining material supply for fishmeal production (Gatlin III et al., 2007; Naylor et al., 2021; Tacon, 2020). Under this scenery, less expensive and highly nutritional alternative protein sources have been evaluated to replace fishmeal in aqua-feeds (Wang et al., 2006). FPH contains high protein levels with balanced amino acids, being one alternative protein source for aqua-feeds (Ospina-Salazar et al., 2016). High contents of short peptides and free amino acids in FPH make diets easily digestible, improve fish growth and feed utilization (Ospina-Salazar et al., 2016). FPH has also been previously reported to enhance the immune response and disease resistance of fish (Hevroy et al., 2005; Ospina-Salazar et al., 2016; Refstie et al., 2004; Siddik et al., 2018b; Xu et al., 2016). Possessing many favorable characteristics, FPH has been used to partially replace fishmeal in diets of many marine fish species (Bui et al., 2014; Chaklader et al., 2020a; Kotzamanis et al., 2007; Tang et al., 2008). However, it has not been a definitive replacement for fishmeal in diets of giant trevally.

MBM is another potential alternative protein source in aqua-feed. MBM contains high protein levels, is rich in trace elements and lacks antinutritional factors (Coutand et al., 2008; Moutinho et al., 2017). Moreover, MBM is always available in large volumes, and its price is relatively lower than fishmeal (Esmaeili et al., 2017). MBM has been partially substituted for fishmeal in the diets of many marine fish without compromising their growth (Abdul Kader et al., 2011; Ai et al., 2006; Esmaeili et al., 2017; Moutinho et al., 2017; Robaina et al., 1997; Tang et al., 2018). However high MBM levels may cause mixed unexpected responses in fish because of their high ash content and less digestibility (Robaina et al., 1997). Therefore, the selection of supplemented nutritional sources to overcome the undesirable characteristics of MBM is imperative to increase the capacity of high MBM levels in fish diets. FPH has been previously reported to effectively reduce the negative impact on the growth and health of fish fed high non-fishmeal protein diets such as plant meal (Costa et al., 2020; Gisbert et al., 2018; Khosravi et al., 2018) and poultry by-product meal (Chaklader et al., 2021; Pham et al., 2021b; Siddik et al., 2019). However, the effects of FPH

supplementation in fish, involving giant trevally fed high MBM diets have yet to be subjected, thus need to be investigated.

Overall, studies on protein and lipid requirement, the use of FPH sources at different inclusion levels to partially replace fishmeal, have been reported in many marine fish, but still unknown in giant trevally. Moreover, FPH can reduce the adverse effects of the plant meal and poultry by-product in aqua-feeds, but published documents have not reported whether FPH can enhance the efficacy of MBM in diets for fish species, including giant trevally. That unknown information should be investigated to contribute to the balanced and cost-effective formulated diets for giant trevally.

CHAPTER 3. MATERIALS AND METHODS

3.1. Ethical statement

All experiments were performed according to the Australian Code of Practice for the care and use of animals for scientific purposes, as approved by Animal Ethics Committee of the Curtin University, Bentley, Australia (Permit Number: ARE2022-15).

3.2. Materials

Juvenile giant trevally, selected from the marine fish hatchery in Nha Trang city, were used for the research. All feeding trials were carried out in the wet laboratory in the hatchery located in Nha Trang, Khanh Hoa province, Vietnam. The research was run from May 2021 to December 2022.

Figure 4: Juvenile giant trevally used for the research.

Figure 5. (a) Cement tanks for acclimating stage; (b) system for experiments.

3.3. Diagram of research

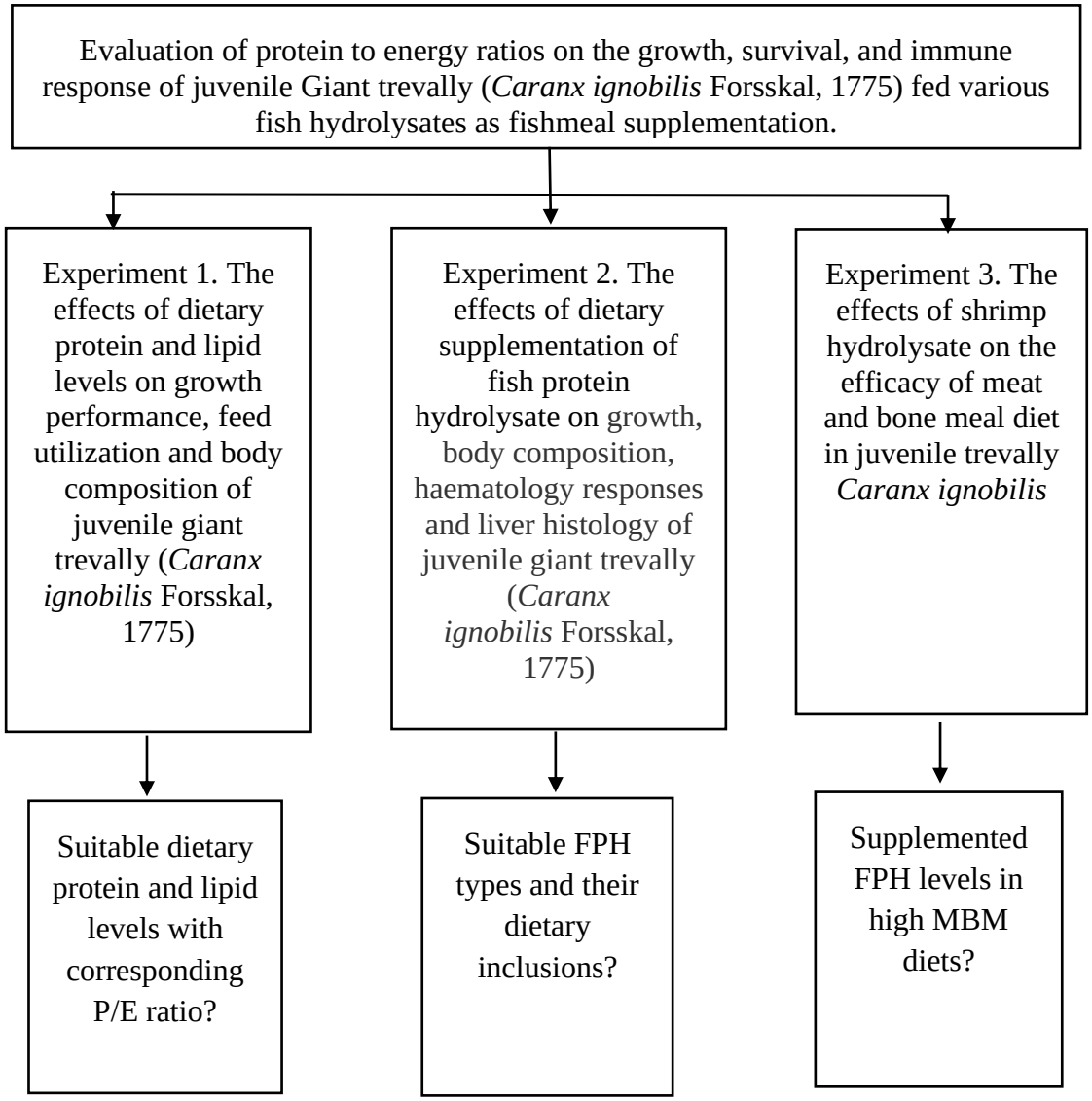


Figure 6. Research diagram.

3.4. Experiment 1. The effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile giant trevally (*Caranx ignobilis* Forsskal, 1775)

3.4.1. Diet preparation

Three dietary protein levels (42%, 47% and 52%) and two lipid levels (10% and 18%) were prepared to produce six experimental diets, labelled as: P42:L10, P42:L18, P47:L10, P47:L18, P52:L10, P52:L18. The corressponding calculated protein to enery ratios for six diets were 20.23 g/MJ, 18.73 g/MJ, 22.31 g/MJ, 20.81 g/MJ, 24.63 g/MJ

and 22.12 g/MJ, respectively. Fishmeal was used as a main protein source. All ingredients were finely ground, weighed and well homogenised. The mixture was then mixed with fish oil and water before pelletising through a 1-2 mm die. The pellets were dried at 60^{o}C in oven for 12 hours and stored at -20^{o}C until use. The ingredient and proximate compositions of the experimental diets were presented in Table 13.

Table 13. Ingredient and analysed chemical composition of experiment diets (g/kg, DM).

Diet code	P42:L10	P42:L18	P47:L10	P47:L18	P52:L10	P52:L18
Ingredient						
Fishmeal	460	460	550	560	640	640
Soybean meal	80	80	80	80	80	80
Wheat gluten	120	120	120	120	120	120
Wheat starch	30	30	30	30	30	30
Fish oil	53	132	45	125	35	115
Cellulose	117	38	45	5	20	0
Vitamin/Mineral	10	10	10	10	10	10
Wheat flour	130	130	120	70	65	5
Proximate composition (%)						
Crude protein	42.07	42.07	47.35	47.36	52.10	51.39
Crude lipid	10.18	18.05	10.27	18.26	10.10	17.90
Carbohydrate	39.57	31.16	34.53	25.16	27.97	23.12
Gross energy (MJ/kg)	20.80	22.46	21.22	22.76	21.15	23.23
Protein/energy ratio (g/MJ)	20.23	18.73	22.31	20.81	24.63	22.12

3.4.2. Experimental procedure

Juvenile giant trevally was bought from a marine fish hatchery located in Nha Trang (Khanh Hoa, Vietnam) and transferred to wet laboratory. During acclimation, fish was fed a commercial diet containing 55% protein and 9% lipid (NRD G8, INVE,

Thailand) for two weeks. After acclimating period, fish (initialy mean weight of 2.12 ± 0.05 g) were randomly allocated into eighteen fiber-glass 250L tanks with 30 individuals/tank. Experiment diets were fed to fish in six groups with 3 replicates for eight weeks. The fish were hand-fed twice a day at 8 am and 4 pm until apparent satiation was achieved. The water in all experimental tanks were managed with flow rate maintained at approximately 5L/min. Water quality parameters, such as temperature, salinity, pH, dissolve oxygen, total ammonia, were maintained in suitable ranges of 29.5 ± 2^{o}C, 31±1 ppt, 7.8 ± 0.2, 6.5 ± 0.3 mg/L and < 0.5 mg/L, respectively.

3.4.3. Sample collection and chemical analyses

At the beginning of the experiment, test diets and 15 fish were sampled for proximate composition analyses. At the termination of feeding trial, fish were anesthetized with MS-222 at 100 mg/L after starving them for 24h. Fish were individually weighed, and length measured using technical scale (FHB 1000, Ningbo Company, China) and fish measuring board with 1mm divisions. Six fish per tank were euthanized with an overdose of MS-222 at >200mg/L before dissecting liver, viscera and muscle to calculate the hepatosomatic and viscera-somatic indexes and amino acids. Another three fish per tank were euthanized for whole-body proximate composition. Proximate composition of feed and fish were determined following (AOAC, 1995). The protein and lipid were measured by Kjeldahl and Soxhlet methods, respectively. Moisture level was calculated after drying the samples in an oven (Thermotec 2000, Contherm Scientific, New Zealand) at 105^{o}C till constant weight was achieved. Ash was analysed by combustion in an electric muffle furnace (Carbolite, Sheffield, UK) at 550^{o}C for 24h. Gross energy was calculated using bomb calorimeter (C2000, IKA, Staufen, Germany). Amino acid profile was analysed by gas chromatography with Biochrom 32 amino acid analyser (Biochrom Ltd, Cambridge, UK) after acid hydrolysis with 6N HCl at 110^{o}C for 24h.

Figure 7. (a) Anesthetizing fish and (b) collecting blood.

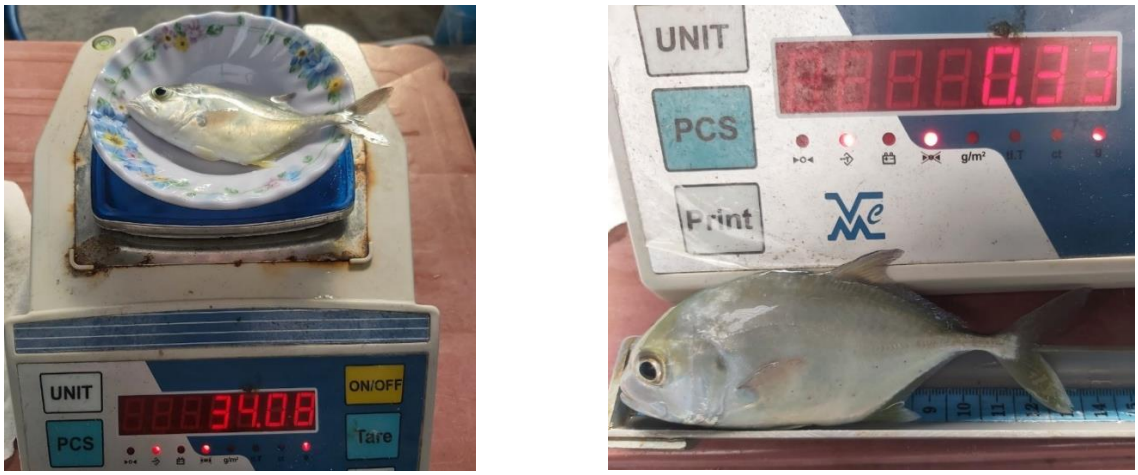


Figure 8. Measuring size and weighting fish.

Figure 9. Sample collection.

3.4.4. Calculations

Performance of fish was determined using the following equations:

$$\text{Survival rate (\%)} = \frac{Final\ animal\ number}{Initial\ animal\ number} \times 100\% \quad (1)$$

$$\text{Specific growth rate (SGR \%/day)} = \frac{Ln\ Final\ body\ wt. - Ln\ Initial\ body\ wt.}{Feeding\ period\ (days)} \quad (2)$$

$$\text{Feed conversion ratio (FCR)} = \frac{Feed\ intake\ in\ dry\ matter\ (g)}{Body\ weight\ gain\ (g)} \quad (3)$$

$$\text{Feed intake (FI, g)} = \frac{Dry\ feed\ consumed}{fish\ number} \quad (4)$$

$$\text{Hepatosomatic index (HSI, \%)} = \frac{Liver\ weight}{Whole\ body\ weight} x100 \quad (5)$$

$$\text{Condition factor (CF, g/cm}^3\text{)} = \frac{Body\ weight}{(Total\ length)^3} x100 \quad (6)$$

$$\text{Viscerasomatic index (VSI, \%)} = \frac{Viscera\ weight}{Whole\ body\ weight} x\ 100 \quad (7)$$

3.4.5. Statistical Analysis

All data were presented as mean ± SD. SPSS for Windows version 22 (IBM, New York, USA) was performed to statistically analyse all the data. The effects of dietary protein, lipid and their interaction on all test parameters of juvenile giant trevally were examined following two-way ANOVA. When a significant interaction between protein and lipid occurred, one-way ANOVA with post hoc Tukey's HSD multiple comparison tests were applied to determine differences among six dietary groups, but not for means of main effects. When no interaction and a significant main effect was detected, a Tukey's in two-way ANOVA was applied to determine the differences among protein diet groups and lipid diet groups. The statistical significance was examined at $P < 0.05$.

3.5. Experiment 2. The effects of dietary supplementation of fish protein hydrolysate on growth, body composition, haematology responses and liver histology of juvenile giant trevally (*Caranx ignobilis* Forsskal, 1775)

3.5.1. Diet preparation

Two hydrolysates, namely SH and TH, were provided by Diana Aquativ (Thailand). The liquid form of TH was centrifuged at 7500 rpm at 4^{o}C for 40 minutes to collect the supernatant. Dried TH contains 61.07% crude protein, 7.8% crude lipid and 9.17% ash. SH powder contains 66.57% crude protein, 8.74% crude lipid and 13.01% ash. The Peruvian fishmeal with 67.05% crude protein, 8.77% crude lipid and 16.17% ash was used as the main protein source in the test diets.

A basal fishmeal-based diet with 52% protein and 10% lipid was produced as a control diet. Six other diets were then formulated by partially replacing the fishmeal with TH and SH at levels of 7% (30 g/kg), 13% (60 g/kg) and 20% (90 g/kg), namely TH30, TH60, TH90, SH30, SH60, SH90. Seven diets were produced using the procedure of Pham et al. (2020). Briefly, all ingredients were finely ground by a grinder (HMB03, Hai Minh Co. Ltd, Vietnam), and then homogenously mixed using a 20QT Mixer (CS200, Taiwan). A laboratory extruder (SV120, Sao Viet Machine Ltd, Vietnam) was used to pelletise the diet through a 2 mm die before air-drying to less than 10g/100g of moisture and stored at -15 ^{o}C until the beginning of the experiment. The diet formulation and chemical composition is shown in Table 14.

Table 14. Dietary formulation and chemical composition of seven test diets.

Ingredients (g/kg)	Control	SH30	SH60	SH90	TH30	TH60	TH90
Fishmeal[a]	450	420	390	360	420.5	391	362
Soymeal[b]	80	80	80	80	80	80	80
Wheat gluten[b]	120	120	120	120	120	120	120
Wheat starch[b]	30	30	30	30	30	30	30
Fish oil[b]	50	50	50	50	50	50	50
Tuna hydrolysate[c]	0	0	0	0	30	60	90
Shrimp hydrolysate[c]	0	30	60	90	0	0	0
Wheat flour[b]	120	120	120	120	119.5	119	118
Vitamin premix[b]	10	10	10	10	10	10	10
Corn gluten[b]	130	130	130	130	130	130	130
CaCO3[d]	2	2	2	2	2	2	2
NaCl[d]	5	5	5	5	5	5	5
Dicalcium Phosphate[d]	3	3	3	3	3	3	3

TOTAL	1000	1000	1000	1000	1000	1000	1000
Proximate composition (% dry matter)							
CP	52.86	52.85	52.84	52.82	52.86	52.85	52.87
Lipid	10.30	10.30	10.30	10.29	10.28	10.27	10.26

[a] Made from SGS del Peru S.A.C
[b] Supplied by LongShing Company, Vietnam
[c] Product of Diana Aquativ, Thailand
[d] Thermo Fisher Scientific, Vietnam

3.5.2. Experimental procedure

Juvenile giant trevally (initial mean weight of 4.12 ± 0.03 g) were provided by a marine fish hatchery in Nha Trang city, Vietnam. During the acclimation period, the juveniles were fed the commercial diet containing 55% crude protein and 9% crude lipid (NRD G8, INVE Ltd, Thailand) diet. Then, fish were randomly distributed into twenty-one 250L tanks in triplicates with 30 individuals per tank. During eight-week feeding trial, fish were fed twice daily at 8 am and 4 pm until apparent satiation. Uneaten feed was collected 30 minutes after feeding and stored in freezer to determine feed intake. The water in tanks were managed with a flow rate of approximately 5L/min. Temperature, salinity, pH, dissolved oxygen, and total ammonia were maintained at normal ranges of 29.5 ± 2 ^{o}C, 31±1 ppt, 7.8 ± 0.2, 6.5 ± 0.3 mg/L and < 0.5 mg/L, respectively.

3.5.3. Sample and chemical analysis

Fish were starved for 24 hours before anaesthetizing them with MS-222 at 0.1g/L, and then individually weight to determine initial and final weights. Six fish per tank were dissected for liver and viscera extractions and for determining hepatosomatic, viscera-somatic index. Whole-body proximate composition was analysed using other three fish per tank. The chemical composition of feed and fish were determined using standard methods (AOAC, 1995). Kjeldahl and Soxhlet methods (AOAC, 1995) were applied to analyse crude proteins and crude lipids, respectively. The whole-body fatty acid composition was analysed following the procedure of O'Fallon et al. (2007).

3.5.4. Haematological parameters

Six fish in each tank were collected blood after being anaesthetized with MS-222 at 100 mg/L. The blood was transferred to non-heparinized tubes and allowed to clot at room temperature for 2h before centrifuging at 5000 g for 10 mins at 4 ^{o}C to separate serum for the analysis of total cholesterol, triglyceride, protein, glucose, albumin, and globulin using an automated blood analyser (Beckman Coulter AU680, USA).

3.5.5. Histological examination

The liver was dehydrated in ethanol, equilibrated in xylene and then put into paraffin wax. The 5-6 µm sections were stained in Hematoxylin-Eosin solution and examined under a light microscopy (Labomed CXR3, LABO America, Inc, USA).

3.5.6. Calculations

The variables in all tanks were calculated using equations described in section 3.4.4.

3.5.7. Statistical Analysis

The data was analysed using SPSS for Windows version 22 (IBM, New York, USA). All data was performed to One way ANOVA followed by Duncan multiple range test to determine the differences among treatments. The quadratic regression was applied to display the correlation between dietary hydrolysate supplementation and SGR of giant trevally. The statistical significance was examined at $P < 0.05$.

3.6. Experiment 3: The effect of shrimp hydrolysate on the efficacy of meat and bone meal diet in juvenile trevally *Caranx ignobilis*

3.6.1. Diet preparation

The fishmeal-based diet with 52% protein and 10% lipid was formulated as a control diet (MBM0) and two other diets were prepared by replacing 25% (MBM25) and 50% (MBM50) fishmeal protein with MBM protein. Another three diets with the same MBM inclusion levels as above were prepared with 45 g/kg SH supplementation (MBM0SH, MBM25SH, and MBM50SH, respectively). All diets were made isonitrogenous and had the same energy values. All the dietary ingredients were finely ground, weighed, and well homogenised. The mixture was then mixed with fish oil and water before pelletising through a 1-2 mm die. The pellets were dried at 60^{o}C in the oven for 12 hours and stored at -20^{o}C until use. The composition of feed

ingredients and experimental diets were presented in Table 15 and Table 16, respectively.

Table 15. Proximate composition of the ingredients used in the preparation of six diets (g/100 g in dry matter).

Ingredients	Crude protein	Total lipid	Ash	Dry matter	Organic matter[e]
Fishmeal[a]	67.05	8.77	16.17	92.32	83.83
Shrimp hydrolysate[b]	66.57	8.74	13.01	95.12	86.99
Meat and bone meal[c]	50.68	4.79	36.52	93.05	63.48
Soybean meal[d]	47.81	0.70	6.77	88.91	92.23
Wheat flour[d]	11.96	1.51	0.31	83.89	99.69
Wheat gluten[e]	75.85	0.53	0.25	91.47	99.75
Starch[d]	n/a	n/a	0.30	86.60	99.70
Fish oil[d]	n/a	99.50	0.29	99.80	99.71

[a] Made from SGS del Peru S.A.C
[b] Product of Diana Aquativ (Thailand)
[c] Supplied by Sunjin Vina Co., Ltd (Vietnam)
[d] Supplied by TomKing Company (Vietnam)
[e] Supplied by LongShing Company (Vietnam)
[f] Organic matter = (100 – ash content)

Table 16. Feed ingredients and approximate compositions of the six test diets.

	MBM0	MBM0SH	MBM25	MBM25SH	MBM50	MBM50SH
Ingredients (g/kg)						
Fishmeal	470	425.3	352.5	307.8	235	190.3
Shrimp hydrolysate	0	45	0	45	0	45
Meat and bone meal	0	0	155.5	155.5	311	311
Soybean meal	90	90	90	90	90	90
Wheat gluten	120	120	120	120	120	120
Wheat starch	30	30	30	30	30	30
Fish oil	50	50	50	50	60	60
Cellulose	100	99.7	62	61.7	14	13.7
Gluten	120	120	120	120	120	120
Vitamin/Mineral	10	10	10	10	10	10

NaCl	5	5	5	5	5	5
$CaHPO_4$	3	3	3	3	3	3
CaCO3	2	2	2	2	2	2
TOTAL	1000	1000	1000	1000		1000
Proximate composition (% dry matter)						
CP	52.49	52.53	52.11	52.64	52.22	51.96
Lipid	10.18	10.06	10.28	9.95	10.52	10.45
Moisture	9.23	9.04	9.35	9.57	9.12	9.43
Ash	10.18	10.06	14.20	14.16	17.97	17.92
Essential amino acids (%)						
Arginine	2.88	2.91	3.03	3.01	3.05	3.02
Histidine	1.14	1.12	1.05	1.02	0.97	1.01
Isoleucine	1.77	1.82	1.45	1.49	1.24	1.25
Leucine	3.44	3.26	2.75	2.79	2.27	2.31
Lysine	2.40	2.22	1.87	1.89	1.36	1.39
Methionine	1.06	1.05	0.94	0.95	0.82	0.84
Phenylalanine	2.02	2.11	1.92	1.95	1.71	1.73
Threonine	2.06	1.94	2.01	2.05	1.92	1.94
Valine	2.20	2.21	2.01	2.05	1.87	1.89

3.6.2. Experimental procedure

Juveniles of giant trevally were supplied by a commercial marine fish hatchery in Nha Trang (Khanh Hoa, Vietnam). The fish were acclimatized to the experimental condition for 2 weeks before feeding trial. During acclimation, fish were fed a commercial diet containing 55% protein and 9% lipid (Inve G8, Inve Aquaculture INC, USA). After acclimation, the uniform-size and healthy fish were selected and randomly distributed into eighteen 250L fiberglass tanks in triplicates with 30 individuals/tank. Fish (initially mean weight of 5.11 ± 0.04 g) were fed in six dietary groups for eight weeks. Fish were hand-fed at 8 am and 4 pm until they achieved apparent satiation. Uneaten feed was collected 30 minutes after feeding and stored in freezer to determine feed intake. The water in tanks were managed with a flow rate of approximately 5L/min. Water quality parameters, including temperature, salinity, pH,

dissolved oxygen, and total ammonia, were maintained in suitable ranges of 29.5 ± 2^{o}C, 31±1 ppt, 7.8 ± 0.2, 6.5 ± 0.3 mg/L and < 0.5 mg/L, respectively.

3.6.3. Sample collection and chemical analyses

At the beginning of the experiment, fifteen fish were sampled to analyse initial proximate composition. At the end of the experiment, fish were starved for 24 hours before anesthetizing them with MS-222 at 100 mg/L, then individually weighed and length measured using technical scale (FHB 1000, Ningbo Company, China). Six fish per tank were anesthetizing for blood collection; then those fish were euthanized with an overdose of MS-222 at >200mg/L before dissecting intestine, liver, viscera and muscle to calculate the hepatosomatic and viscera somatic indexes and take histological examination. Other three fish in each tank were euthanized for tissue proximate composition. Proximate composition of feed and fish were determined following (AOAC, 1995). The protein and lipid were analysed using Kjeldahl and Soxhlet methods, respectively. Moisture level was determined by drying in an oven (Thermotec 2000, Contherm Scientific, New Zealand) at 105^{o}C. Ash was analysed by combustion at 550^{o}C for 24h in an electric muffle furnace (Carbolite, Sheffield, UK). Amino acids in the diets were determined after acid hydrolysis by gas chromatography with GC 2010 Plus (Shimadzu, Kyoto, Japan). The muscle fatty acid composition was analysed following the method described by O'Fallon et al. (2007).

3.6.4. Haematological parameters

As mentioned in the section above, six fish in each tank were anaesthetized for blood collection by puncturing their caudal vein with a 25-gauge needle, as the method described by Pham et al. (2019). The blood was centrifuged at 5000 g for 10 mins at 4 ^{o}C, then separated serum was analysed for total cholesterol, triglyceride, protein, glucose using an automated blood analyser (Beckman Coulter AU680, USA).

3.6.5. Histological examination

After collecting blood, those six fish were euthanized to dissect intestines and livers. The intestines and livers were put into a 10% buffer formalin solution. The livers were dehydrated in ethanol, equilibrated in xylene and then embedded in paraffin wax following standard histological techniques. The 5-6 µm sections were cut and stained

with hematoxylin-eosin solution and examined under light microscopy (Labomed CXR3, USA) and imaged with 10M camera. The number of goblet cells were counted in the highest 10 mucosal folds with the numbers expressed as average number of goblet cells per fold as described by Ramos et al. (2017).

3.6.6. Calculations

The variables in all tanks were calculated using equations described in section 3.4.4.

3.6.7. Statistical Analysis

All data were presented as mean ± SE. SPSS for Windows version 22 (IBM, New York, USA) was used to perform statistical analysis of all the data sets. The effects of dietary MBM inclusion levels, SH supplementation and their interaction on all test parameters of juvenile giant trevally were examined following two-way ANOVA. When a significant interaction between dietary treatments occurred, one-way ANOVA with post hoc Tukey's HSD multiple comparison test was applied to determine differences among six dietary groups, but not for means of main effects. When no interaction and a significant main effect was detected, a Tukey's in two-way ANOVA was applied to determine the differences among substituted MBM groups. The statistical significance was examined at $P < 0.05$.

CHAPTER 4. RESULTS

4.1. Experiment 1: Effects of dietary protein and lipid levels on growth performance, feed utilization and body composition of juvenile giant trevally (*Caranx ignobilis* Forsskal, 1775)

4.1.1. Growth performance and feed utilization

Growth performance and feed utilization are represented in Table 17. Total length (TL), final body weight (FBW) and specific growth rate (SGR) were significantly ($P < 0.05$) affected by the interaction between protein and lipid. The highest ($P < 0.05$) growth rate in terms of TL, FBW and SGR were achieved in fish fed the P52:L10 diet, corresponding with P/E ratio of 24.63 g/MJ; followed by the P52:L18 diet.

Dietary protein significantly ($P < 0.05$) affected the feed intake (FI) and feed conversion ratio (FCR) of giant trevally. Fish fed high protein diets gained more higher ($P < 0.05$) FI and lower FCR than fish fed low protein diets. High survival rate was observed in all diets and was not significantly ($P > 0.05$) influenced by dietary protein, lipid level and their interaction. The result stated that the best growth performance of fish achieved in P52:L10 diet with P/E ratio of 24.63 g/MJ. Regardless lipid contents, 52% protein performed the best feed utilization.

Table 17. Growth performance and feed utilization of giant trevally fed test diets.

Diet	TL (cm)	FBW (g/fish)	SGR (%/day)	FI (g/fish)	FCR	Survival (%)
P42:L10	10.04 ± 0.08^{a}	14.58 ± 0.03^{a}	3.44 ± 0.01^{a}	18.28 ± 0.42	1.25 ± 0.03	92.22 ± 1.11
P42:L18	10.37 ± 0.03^{ab}	15.30 ± 0.13^{a}	3.54 ± 0.02^{ab}	18.63 ± 0.46	1.21 ± 0.03	92.22 ± 2.22
P47:L10	10.66 ± 0.09^{bc}	16.79 ± 0.15^{b}	3.69 ± 0.02^{c}	18.58 ± 0.49	1.11 ± 0.03	92.22 ± 1.11
P47:L18	10.37 ± 0.14^{ab}	15.91 ± 0.62^{ab}	3.60 ± 0.07^{bc}	17.16 ± 1.23	1.08 ± 0.04	94.44 ± 1.11
P52:L10	11.36 ± 0.04^{d}	20.43 ± 0.13^{d}	4.05 ± 0.01^{e}	20.36 ± 0.72	1.00 ± 0.04	95.56 ± 1.11
P52:L18	10.94 ± 0.07^{c}	18.47 ± 0.38^{c}	3.86 ± 0.04^{d}	20.55 ± 0.60	1.11 ± 0.04	95.56 ± 2.94
Means of main effects of dietary protein level						
P42	10.21	14.94	3.49	18.45^{A}	1.23^{B}	92.22

P47	10.52	16.35	3.65	17.87^{A}	1.09^{A}	93.33
P52	11.15	19.45	3.96	20.45^{B}	1.06^{A}	95.56
Means of main effects of dietary lipid level						
L10	10.69	17.27	3.73	19.07	1.12	93.33
L18	10.56	16.56	3.67	18.78	1.13	94.07
Two-way ANOVA: P values						
Protein	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	NS
Lipid	NS	< 0.05	NS	NS	NS	NS
P x L	< 0.05	< 0.05	< 0.05	NS	NS	NS

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate the significant differences ($P < 0.05$) among means of the main effects of protein level. TL final total length; FBW Final body weight; SGR Specific growth rate; FI Feed intake; FCR Feed conversion ratio.

4.1.2. Proximate composition and somatic index

The biochemical composition and somatic index of the whole body of juvenile giant trevally are displayed in Table 18. Moisture and ash were not ($P > 0.05$) affected by any treatment. Crude protein of whole body increased ($P < 0.05$) in fish fed high protein diets. The same trend was recorded when dietary lipid increased. The interaction between the dietary protein and lipid showed the significant ($P < 0.05$) effect on lipid level of whole body, where high lipid was obtained in fish fed high dietary protein and lipid. Dietary protein, lipid, and their interaction did not ($P > 0.05$) affect viscerasomatic index (VSI) and condition Factor (CF), however hepatosomatic index (HSI) decreased with increasing lipid from 10% to 18%.

Table 18. Whole body composition (%) and somatic index (%) of giant trevally fed test diets.

Diet	Crude protein	Crude lipid	Moisture	Ash	VSI	HSI	CF (g/cm^3)
P42:L10	17.86 ± 0.30	4.39 ± 0.04[a]	71.97 ± 1.00	5.17 ± 0.05	5.88 ± 0.13	1.59 ± 0.09	1.45 ± 0.03
P42:L18	18.45 ± 0.14	4.65 ± 0.08[a]	71.19 ± 0.97	5.03 ± 0.14	5.75 ± 0.07	1.25 ± 0.05	1.38 ± 0.04
P47:L10	19.58 ± 0.24	4.50 ± 0.07[a]	70.47 ± 0.29	5.27 ± 0.05	5.45 ± 0.14	1.30 ± 0.03	1.40 ± 0.03
P47:L18	20.17 ± 0.36	5.49 ± 0.09[b]	70.80 ± 0.35	5.06 ± 0.11	5.38 ± 0.22	1.21 ± 0.02	1.44 ± 0.01
P52:L10	19.61 ± 0.33	5.12 ± 0.05[b]	70.88 ± 0.61	5.17 ± 0.04	5.52 ± 0.21	1.38 ± 0.29	1.39 ± 0.01
P52:L18	21.19 ± 0.45	6.17 ± 0.18[c]	70.41 ± 0.33	4.62 ± 0.19	6.14 ± 0.35	1.05 ± 0.10	1.41 ± 0.02
Means of main effects of dietary lipid level							
P42	18.16[A]	4.52	71.58	5.10	5.82	1.42	1.41
P47	19.88[B]	5.00	70.64	5.16	5.41	1.26	1.42
P52	20.40[B]	5.65	70.65	4.89	5.83	1.21	1.40
Means of main effects of dietary lipid level							
L10	19.02[X]	4.67	71.11	5.20[Y]	5.62	1.42	1.41
L18	19.94[Y]	5.44	70.70	4.90[X]	5.76	1.17	1.41
Two-way ANOVA: P values							

Protein	< 0.05	< 0.05	NS	NS	NS	NS	NS
Lipid	< 0.05	< 0.05	NS	< 0.05	NS	< 0.05	NS
P x L	NS	< 0.05	NS	NS	NS	NS	NS

Values are means of 3 replicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate the significant differences ($P < 0.05$) among means of the main effects of protein level. ($P < 0.05$). Means with different uppercase alphabets (X, Y) within a column indicate the significant difference ($P < 0.05$) among means of the main effects of lipid level. VSI Viscera-somatic index; HSI Hepatosomatic index; CF Condition factor.

4.1.3. Amino acid profile in fish body

Non-essential amino acid profiles of the fish are shown in Table 19. Non-essential amino acids were not ($P > 0.05$) affected by any dietary lipid, except significantly ($P < 0.05$) lower alanine and glutamine in fish fed high lipid diets were observed. In contrast, high dietary protein led to increased levels of alanine, aspartic acid, glutamine and serine. Significant effects in the combination between dietary protein and lipid were observed in glycine and tyrosine.

Table 20 presents the essential amino acid profile of the giant trevally. Except for histidine and phenylamine, other essential amino acids were significantly ($P < 0.05$) affected by the dietary protein, lipid or their interaction. The levels of leucine, valine and arginine increased with the increase in dietary protein level. Whereas leucine, valine and arginine were reduced as dietary lipid increased from 10% to 18%. The interaction affected isoleucine, lysine, threonine, and methionine and were at highest ($P < 0.05$) levels in fish fed P52:L10.

Table 19. Non-essential amino acid profile of juvenile giant trevally fed test diets.

Diet	Alanine	Aspartate	Glutamate	Glycine	Proline	Serine	Tyrosine
P42:L10	0.76 ± 0.03	1.75 ± 0.03	2.68 ± 0.03	1.02 ± 0.02^{b}	0.65 ± 0.02	0.67 ± 0.04	0.70 ± 0.01^{a}
P42:L18	0.68 ± 0.02	1.68 ± 0.04	2.44 ± 0.05	0.92 ± 0.01^{a}	0.66 ± 0.02	0.69 ± 0.01	0.87 ± 0.04^{b}
P47:L10	0.73 ± 0.01	1.78 ± 0.01	2.83 ± 0.06	0.95 ± 0.01^{ab}	0.67 ± 0.02	0.71 ± 0.01	0.71 ± 0.01^{a}
P47:L18	0.73 ± 0.02	1.74 ± 0.04	2.78 ± 0.10	0.96 ± 0.02^{ab}	0.68 ± 0.02	0.69 ± 0.003	0.72 ± 0.01^{a}
P52:L10	0.84 ± 0.03	2.01 ± 0.04	3.30 ± 0.01	1.00 ± 0.02^{b}	0.66 ± 0.01	0.79 ± 0.02	0.78 ± 0.02^{a}
P52:L18	0.79 ± 0.01	1.97 ± 0.02	3.11 ± 0.11	0.98 ± 0.01^{ab}	0.64 ± 0.02	0.74 ± 0.01	0.70 ± 0.01^{a}
Means of main effects of dietary protein level							
P42	0.72^{A}	1.72^{A}	2.56^{A}	0.97	0.66	0.68^{A}	0.78
P47	0.73^{A}	1.76^{A}	2.81^{B}	0.96	0.68	0.70^{A}	0.72
P52	0.82^{B}	1.99^{B}	3.20^{C}	0.99	0.65	0.77^{B}	0.74
Means of main effects of dietary lipid level							
L10	0.78^{Y}	1.84	2.93^{Y}	0.99	0.66	0.72	0.73
L18	0.73^{X}	1.80	2.78^{X}	0.95	0.66	0.71	0.76
Two-way ANOVA: P values							

Protein	< 0.05	< 0.05	< 0.05	NS	NS	< 0.05	< 0.05
Lipid	< 0.05	NS	< 0.05	< 0.05	NS	NS	NS
P x L	NS	NS	NS	< 0.05	NS	NS	< 0.05

Values are means of 3 replicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate the significant differences ($P < 0.05$) among means of the main effects of dietary protein level. ($P < 0.05$). Means with different uppercase alphabets (X, Y) within a column indicate the significant difference ($P < 0.05$) among means of the main effects of dietary lipid level.

Table 20. Essential amino acid profile of juvenile giant trevally fed test diets.

Diet	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Valine	Arginine
P42:L10	0.60 ± 0.01	0.79 ± 0.03^{a}	1.31 ± 0.05	1.25 ± 0.05^{ab}	0.65 ± 0.01^{a}	0.84 ± 0.02	0.82 ± 0.01^{b}	0.88 ± 0.03	1.04 ± 0.02
P42:L18	0.55 ± 0.01	0.74 ± 0.03^{a}	1.15 ± 0.01	1.16 ± 0.03^{a}	0.64 ± 0.01^{a}	0.83 ± 0.05	0.66 ± 0.02^{a}	0.76 ± 0.02	0.94 ± 0.02
P47:L10	0.61 ± 0.01	0.81 ± 0.02^{ab}	1.38 ± 0.02	1.44 ± 0.02^{c}	0.70 ± 0.01^{a}	0.81 ± 0.01	0.84 ± 0.01^{b}	0.92 ± 0.01	1.07 ± 0.01
P47:L18	0.61 ± 0.003	0.77 ± 0.02^{a}	1.33 ± 0.01	1.36 ± 0.04^{bc}	0.67 ± 0.01^{a}	0.86 ± 0.03	0.87 ± 0.04^{b}	0.90 ± 0.01	1.03 ± 0.02
P52:L10	0.54 ± 0.04	0.88 ± 0.01^{b}	1.51 ± 0.02	1.77 ± 0.04^{d}	0.80 ± 0.02^{b}	0.80 ± 0.01	0.91 ± 0.06^{b}	1.01 ± 0.04	1.21 ± 0.04
P52:L18	0.51 ± 0.06	0.79 ± 0.02^{a}	1.40 ± 0.02	1.50 ± 0.02^{c}	0.69 ± 0.01^{a}	0.84 ± 0.02	0.83 ± 0.01^{b}	0.89 ± 0.04	1.08 ± 0.03

Means of main effects of dietary protein level

P42	0.58	0.77	1.23^{A}	1.20	0.64	0.84	0.74	0.82^{A}	0.99^{A}
P47	0.61	0.79	1.35^{B}	1.40	0.69	0.83	0.86	0.91^{B}	1.05^{B}
P52	0.53	0.83	1.45^{C}	1.64	0.74	0.82	0.87	0.95^{B}	1.14^{C}
Means of main effects of dietary lipid level									
L10	0.58	0.83	1.40^{Y}	1.49	0.72	0.82	0.86	0.94^{Y}	1.11^{Y}
L18	0.56	0.76	1.29^{X}	1.34	0.66	0.84	0.79	0.85^{X}	1.01^{X}
Two-way ANOVA: P values									
Protein	NS	< 0.05	< 0.05	< 0.05	< 0.05	NS	< 0.05	< 0.05	< 0.05
Lipid	NS	< 0.05	< 0.05	< 0.05	< 0.05	NS	< 0.05	< 0.05	< 0.05
P x L	NS	< 0.05	NS	< 0.05	< 0.05	NS	< 0.05	NS	NS

Values are means of 3 replicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate the significant differences ($P < 0.05$) among means of the main effects of dietary protein level. ($P < 0.05$). Means with different uppercase alphabets (X, Y) within a column indicate the significant difference ($P < 0.05$) among means of the main effects of dietary lipid level.

4.2. Experiment 2. The effects of dietary supplementation of fish protein hydrolysate on growth, body composition, haematology responses and liver histology of juvenile giant trevally (*Caranx ignobilis* Forsskal, 1775)

4.2.1. Growth performance, feed utilisation and somatic indices

The higher (P = 0.005) final body weight (FBW) and specific growth rate (SGR) were achieved in fish fed SH30, SH60, TH30 and TH60 than the control, whereas no differences in FBW and SGR were observed among the fish fed SH90, TH90 and control (Table 21). Based on quadratic regression, the optimum dietary SH and TH in diets for juvenile giant trevally were 47.67 and 48.46 g/kg respectively (Figure 10).

Table 21. Growth performance and feed utilisation of juvenile giant trevally fed test diets.

Diets	FBW (g/fish)	SGR (%/day)	FCR	FI (g/fish)	Survival (%)
Control	20.53 ± 0.49^{a}	2.86 ± 0.04^{a}	1.67 ± 0.01^{c}	27.33 ± 0.88	89.33 ± 2.67
SH30	23.27 ± 0.19^{c}	3.09 ± 0.01^{c}	1.52 ± 0.03^{a}	29.00 ± 0.58	90.67 ± 1.33
SH60	22.42 ± 0.09^{bc}	3.02 ± 0.00^{bc}	1.51 ± 0.01^{a}	27.67 ± 0.33	89.33 ± 2.67
SH90	21.39 ± 0.66^{ab}	2.93 ± 0.06^{ab}	1.64 ± 0.04^{bc}	28.33 ± 0.88	89.33 ± 1.33
TH30	22.35 ± 0.11^{bc}	3.02 ± 0.01^{bc}	1.50 ± 0.04^{a}	27.33 ± 0.88	86.67 ± 1.33
TH60	22.16 ± 0.37^{bc}	3.00 ± 0.03^{bc}	1.50 ± 0.03^{a}	27.00 ± 0.58	89.33 ± 2.67
TH90	21.20 ± 0.47^{ab}	2.92 ± 0.04^{ab}	1.56 ± 0.03^{ab}	26.67 ± 1.20	89.33 ± 1.33
P-value	0.005	0.005	0.006	0.467	0.896

Values are presented as mean of triplicate groups ± SE (df1, df2: 6,14). Different lowercase alphabets (a, b, c) in the same row indicate significant differences ($P < 0.05$) among treatments. FBW: Final body weight; SGR: Specific growth rate; FCR: Feed conversion ratio; FI: Feed intake.

Fish fed SH30, SH60, TH30 and TH60 showed lower feed conversion ratio (FCR) than fish fed SH90 and the control but were similar to the fish fed TH90. The feed intake (FI) and survival were not different among dietary treatments.

No diet could alter the condition factor (CF) and hepatosomatic indices (HSI) of fish, whereas viscera somatic index (VSI) was affected by FPH. Lower VSI was observed in fish fed SH60 and TH60 than control, SH30 and TH30, whereas fish fed SH90 and TH90 showed no difference from other dietary treatments (Figure 11).

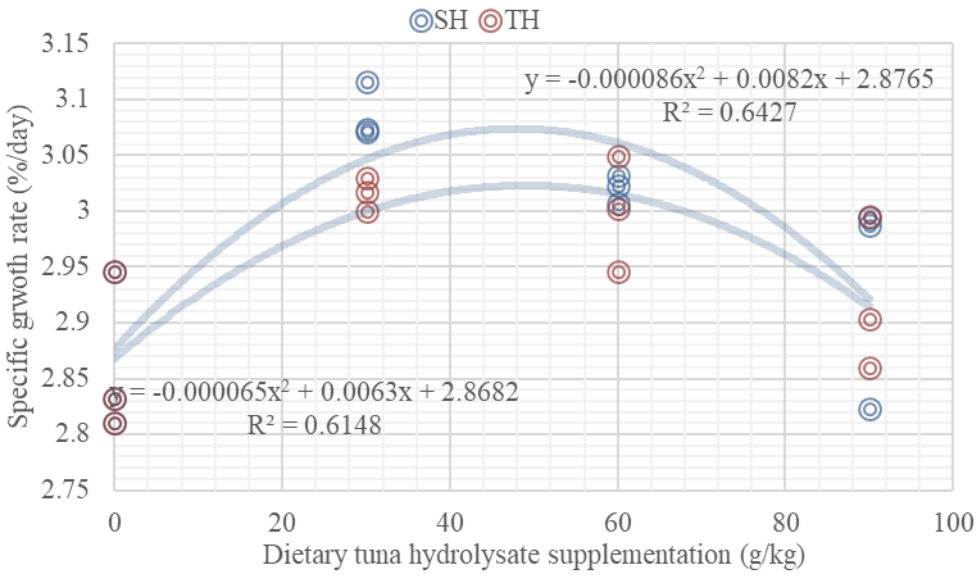


Figure 10. The quadratic regression among specific growth rate (SGR) of juvenile giant trevally and dietary FPH supplementation.

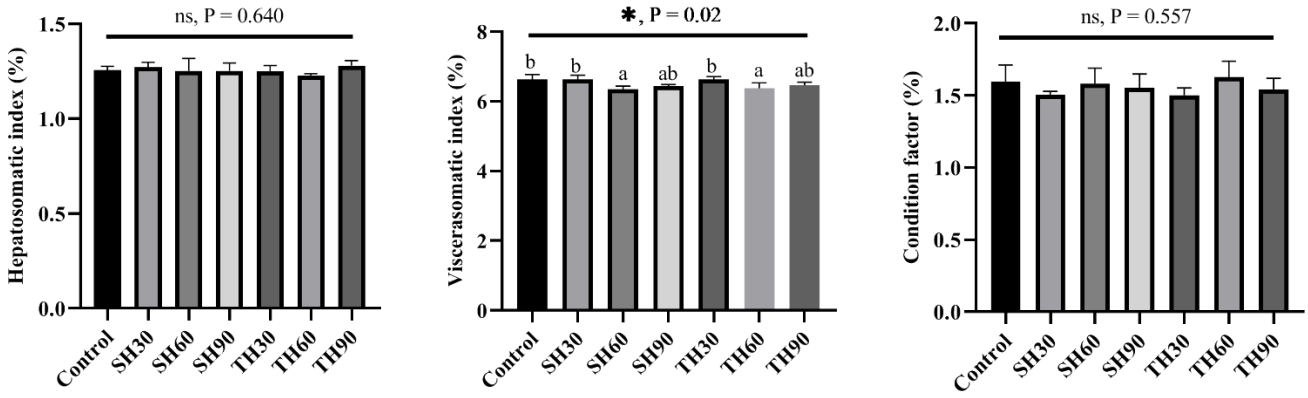


Figure 11. Somatic indexes and condition factor of giant trevally fed experimental diets for 8 weeks (df1, df2: 6,14).

4.2.2. Chemical composition

Whole-body protein, ash and moisture of giant trevally were unaffected by the dietary inclusion of FPH, while lipid content was changed. Highest whole-body lipid was recorded in fish fed TH90, followed by TH60, while no difference was observed due to the dietary treatments of SH30, SH60, SH90, TH30 and control (Figure 12).

The concentrations of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA), particularly 20:5n-3 (EPA) and 22:6n-3 (DHA) were not affected by FPH supplementation.

Lowest (P = 0.040) ∑n-3PUFA level was observed in TH30, while no difference in this content was observed among SH30, SH60, SH90, TH60, TH90 and control (Table 22).

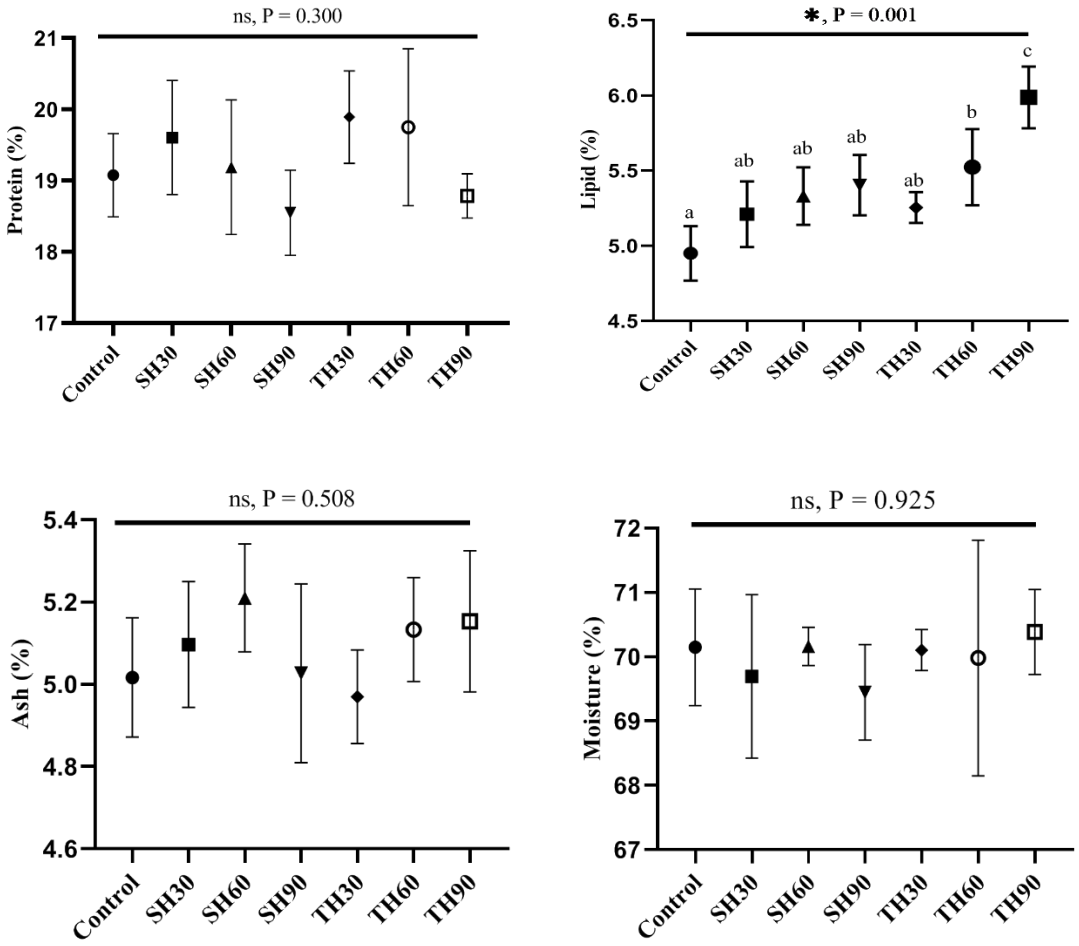


Figure 12. Proximate composition in the whole body of juvenile giant trevally fed tested diets for 8 weeks; ns: non-significant; different subscript letters indicate the difference among treatments.

Table 22. Fatty acid composition (% total fatty acids) in the whole body of giant trevally fed the test diets.

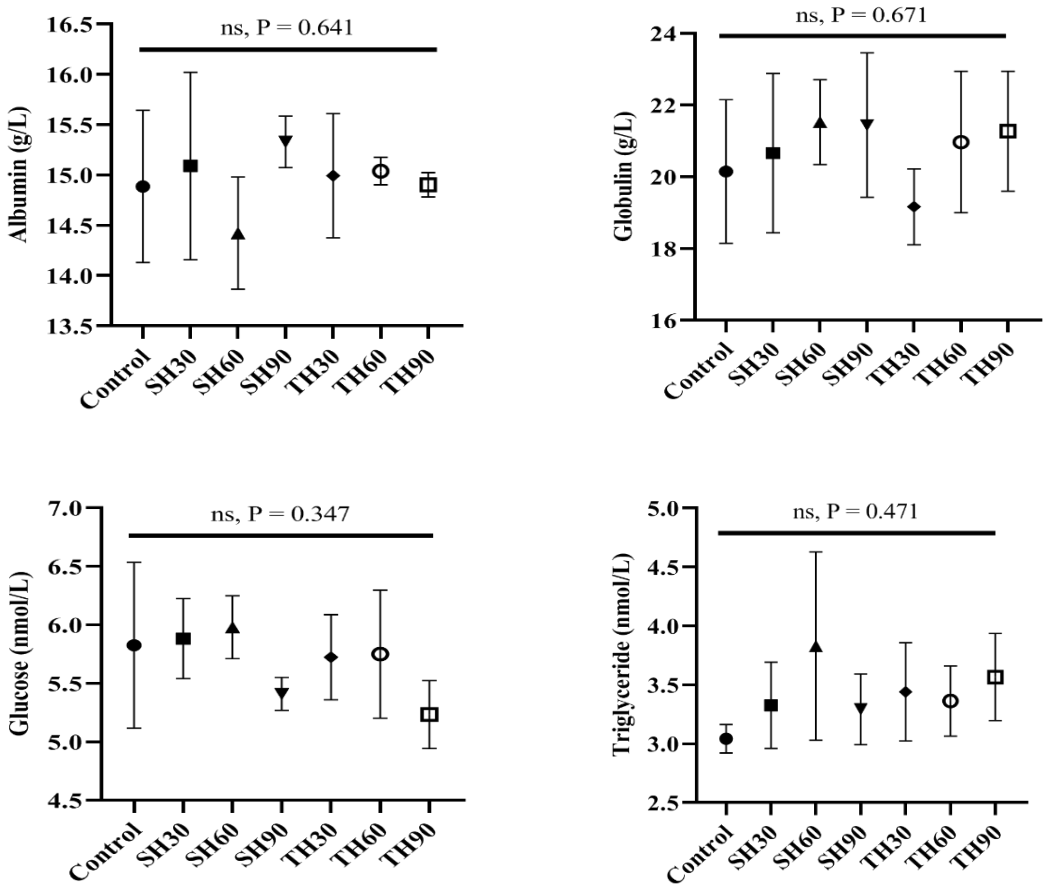
Parameter	Control	SH30	SH60	SH90	TH30	TH60	TH90	*SEM*	*P-value*
14:0	2.32	2.42	2.42	2.54	2.38	2.43	2.70	0.05	0.443
15:0	0.49	0.54	0.39	0.55	0.38	0.47	0.49	0.03	0.578
16:0	19.89	19.66	19.56	19.32	19.56	19.58	19.36	0.22	0.997
18:0	6.97	7.43	7.39	7.12	7.58	7.43	7.34	0.07	0.296

20:0	0.35	0.40	0.39	0.41	0.38	0.41	0.42	0.01	0.582
21:0	1.27^{b}	1.21^{ab}	1.18^{ab}	1.23^{ab}	1.13^{a}	1.22^{ab}	1.25^{ab}	0.01	0.029
22:0	0.21^{b}	0.20^{ab}	0.20^{ab}	0.21^{ab}	0.19^{a}	0.20^{ab}	0.21^{ab}	0.00	0.029
24:0	0.21^{b}	0.20^{ab}	0.20^{ab}	0.21^{ab}	0.19^{a}	0.20^{ab}	0.21^{ab}	0.00	0.029
∑SFA	31.70	32.05	31.73	31.58	31.78	31.93	31.96	0.20	0.998
16:1	3.93	3.95	4.06	4.11	3.94	3.91	4.08	0.04	0.670
17:1	0.28	0.20	0.33	0.55	0.25	0.34	0.35	0.03	0.149
18:1n-9	21.29	22.37	22.32	21.98	22.03	21.60	21.30	0.16	0.348
20:1	2.60	2.68	2.95	3.01	3.45	3.31	3.39	0.10	0.065
22:1	3.44	3.14	3.47	3.49	3.52	3.38	3.40	0.05	0.462
24:1	0.63^{a}	0.60^{a}	0.79^{ab}	0.62^{a}	0.75^{ab}	0.61^{a}	1.03^{b}	0.04	0.029
∑MUFA	33.07	33.14	34.10	33.97	34.13	33.35	33.76	0.17	0.471
18:2n-6	10.12	10.18	10.15	10.34	10.65	10.87	10.60	0.09	0.141
18:3n-3	2.68^{b}	2.74^{b}	2.88^{b}	1.85^{a}	1.70^{a}	1.62^{a}	1.74^{a}	0.13	≤0.001
18:3n-6	0.42^{c}	0.40^{bc}	0.39^{b}	0.21^{a}	0.19^{a}	0.20^{a}	0.21^{a}	0.02	0.001
20:5n-3	5.20	5.02	5.04	5.28	5.00	5.06	4.99	0.05	0.698
22:6n-3	14.74	14.17	13.87	14.45	13.97	14.86	14.40	0.11	0.074
∑PUFA	34.00	33.32	33.11	32.95	32.26	33.44	32.76	0.20	0.381
∑n-3 PUFA	22.83^{b}	22.13^{ab}	21.98^{ab}	21.79^{ab}	20.86^{a}	21.75^{ab}	21.33^{ab}	0.18	0.040
∑n-6 PUFA	10.75	10.78	10.74	10.75	11.03	11.28	11.01	0.08	0.500
n-3/n-6 PUFA	2.12^{b}	2.05^{ab}	2.04^{ab}	2.03^{ab}	1.89^{a}	1.93^{ab}	1.94^{ab}	0.02	0.019
n-3 HUFA	19.94	19.19	18.91	19.73	18.97	19.93	19.39	0.12	0.063

Values are presented as the mean of triplicate groups (df1, df2: 6,14). SEM: standard error of means. Different lowercase alphabets (a, b, c) in the same row indicate significant differences ($P < 0.05$) among treatments. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; HUFA: highly unsaturated fatty acids.

4.2.3. Haematological parameters

Haematological parameters were not affected by FPH supplementation except for cholesterol and total protein. The cholesterol of fish fed TH90 was lower than the fish fed control and SH30 diets but did not differ from other dietary groups (P = 0.011). Total protein in fish fed control, SH30, SH60 and TH30 was significantly higher than TH90, while no changes in total protein were seen among fish fed SH30, SH60, SH90, TH30 and TH60 (Figure 13).



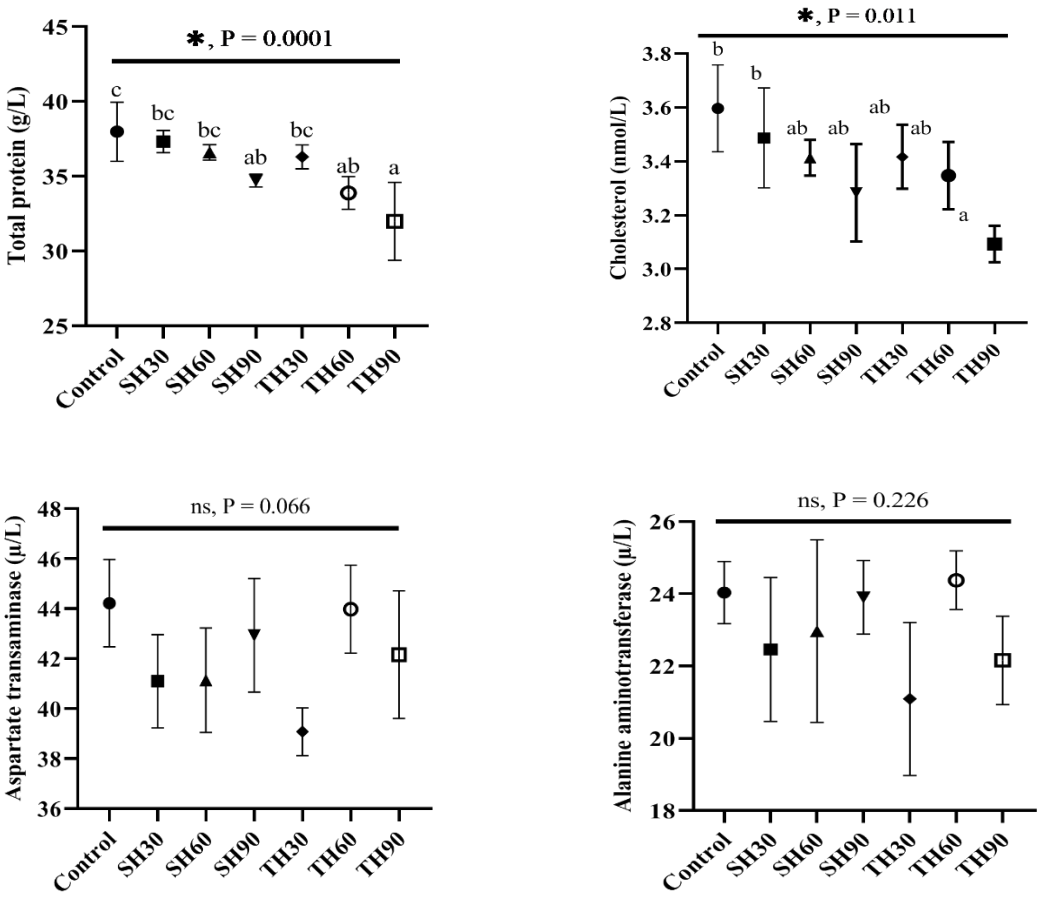
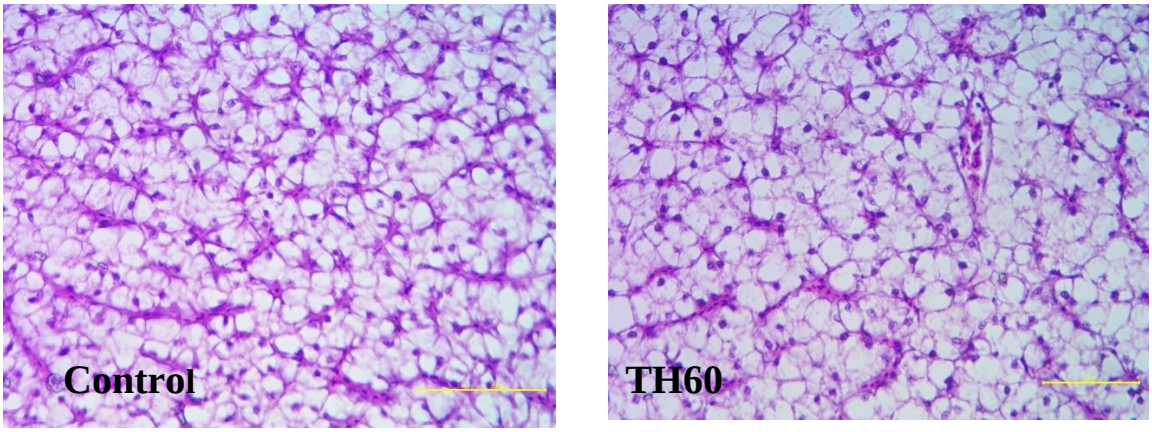


Figure 13. Haematological and serum biochemical parameters of giant trevally fed experimental diets for 8 weeks. ns: non-significant; different subscript letters indicate the difference among treatments.

4.2.4. Histological examination



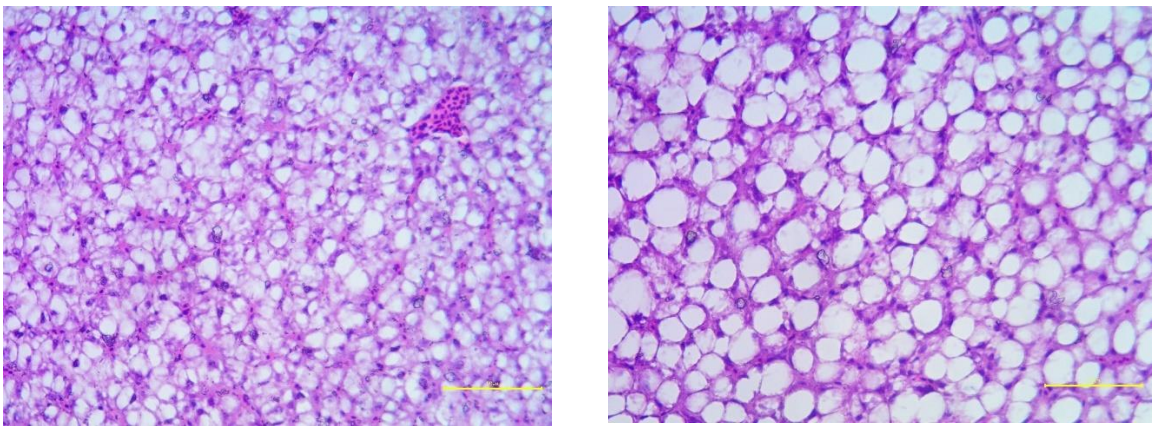


Figure 14. Liver microscopic of giant trevally fed FPH for eight weeks (scale bar = 50 µm, at 400 magnification).

Histopathological observation showed that increased lipid droplets occurred in hepatocytes of fish fed TH90, whereas normal cells were recorded in the juveniles fed the remaining diets (Figure 14).

4.3. Experiment 3: The effect of shrimp hydrolysate on the efficacy of meat and bone meal diet in juvenile trevally *Caranx ignobilis*

4.3.1. Growth performance and feed utilisation

The fish was fed slowly until satiation, and very few residual feeds at the tank's bottom indicated the fish accepted experimental diets. No pathological signs were observed during the feeding trial. After 8 weeks, dietary MBM inclusion levels, SH supplementation and their interaction significantly influenced the specific growth rate (SGR). The highest ($P<0.05$) SGR was observed in fish fed MBM0SH, followed by MBM0, MBM25SH, MBM50SH while fish fed MBM25 and MBM50 had the lowest SGR (Table 23).

Similarly, feed conversion ratio (FCR) was affected by dietary MBM, SH supplementation and their interaction ($P<0.05$). MBM50 showed higher FCR than MBM0, while no difference in FCR was seen among MBM0, MBM0SH, MBM25 and MBM25SH. Replacing 50% of fishmeal with MBM showed lower FI than fishmeal based-diet while no change in FI was seen among the substitution level of 25% and fishmeal based-diet. Fish fed SH supplemented diets achieved higher FI than fish fed non-SH diets. The highest ($P<0.05$) survival rate was observed in fish fed MBM0 and MBM0SH, followed by MBM25, MBM25SH, MBM50SH, while fish fed MBM50 obtained the lowest survival rate (Table 23).

Table 23. Growth performance and feed utilisation of giant trevally fed test diets.

Diets	FBW (g/fish)	SGR (%/day)	FI (g/fish)	FCR	Survival (%)
MBM0	24.18 ± 0.38	2.78 ± 0.03^{c}	29.67 ± 0.67	1.56 ± 0.03^{ab}	93.33 ± 1.33^{c}
MBM0SH	28.77 ± 1.25	3.08 ± 0.08^{d}	35.00 ± 1.53	1.48 ± 0.02^{a}	89.33 ± 1.33^{bc}
MBM25	20.73 ± 0.29	2.50 ± 0.03^{ab}	27.00 ± 0.58	1.73 ± 0.01^{b}	85.33 ± 1.33^{b}
MBM25SH	25.24 ± 0.92	2.85 ± 0.07^{c}	31.67 ± 2.03	1.57 ± 0.03^{ab}	85.33 ± 1.33^{b}
MBM50	18.40 ± 0.16	2.29 ± 0.02^{a}	26.00 ± 1.00	1.96 ± 0.05^{c}	76.00 ± 2.31^{a}
MBM50SH	22.24 ± 1.08	2.62 ± 0.09^{bc}	28.00 ± 1.73	1.64 ± 0.06^{ab}	85.33 ± 1.33^{b}
Means of main effects of fishmeal protein replacement level					
0	26.47^{C}	2.93	32.33^{B}	1.52	91.33
25	22.98^{B}	2.68	29.33^{AB}	1.65	85.33
50	20.32^{A}	2.46	27.00^{A}	1.80	80.67
Means of main effects of dietary SH supplementation					
0	21.10^{X}	2.52	27.56^{X}	1.75	84.89
45	25.42^{Y}	2.85	31.56^{Y}	1.56	86.67
Two-way ANOVA: P values					
MBM	< 0.05	< 0.05	< 0.05	<0.05	<0.05
SH	< 0.05	< 0.05	< 0.05	<0.05	NS
MBM x SH	NS	< 0.05	NS	<0.05	<0.05

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate significant differences (P < 0.05) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate the significant difference (P < 0.05) among means of the main effects of MBM level. Means with different uppercase alphabets (X, Y) within a column indicate the significant difference (P < 0.05) among means of the main effects of SH supplementation. FBW Final body weight; SGR Specific growth rate; FI Feed intake; FCR Feed conversion ratio.

4.3.2. Proximate composition in muscle and somatic index

Protein and moisture levels in muscle were not different among fish fed any diets. In contrast, lipid content significantly decreased (P<0,05) with increasing dietary MBM levels; it was higher in fish fed diets with SH than groups fed diets without SH. Dietary MBM

affected ash level in muscle as higher ash was recorded in fish fed MBM diets than non-MBM diets (Table 24).

Dietary MBM significantly affected visceral somatic index (VSI) ($P<0.05$), wherein diets with 50% of fishmeal replaced by MBM showed higher VSI in fish than other feeding diets. The interaction between dietary MBM inclusion and SH supplementation affected hepatosomatic index (HSI). Higher HSI was achieved in fish fed MBM0, MBM0SH, MBM25SH compared to fish fed MBM25, MBM50 and MBM50SH. CF was not affected by dietary MBM, SH supplementation and their interaction (Table 24).

SFA and MUFA were affected by MBM levels, supplemented SH and their interaction ($P<0.05$). The lowest SFA and MUFA was obtained in fish fed MBM25SH, whereas no difference was seen among other treatments (Table 25). Replacing fishmeal with MBM decreased PUFA, n-3 PUFA, n-3/n-6 HUFA and n-3 HUFA. On the contrary, SH supplementation improved n-3 PUFA, n-3/n-6 HUFA and n-3 HUFA (Table 26).

Table 24. Muscle composition (%) and somatic index (%) of giant trevally fed test diets.

Diets	Crude protein	Crude lipid	Moisture	Ash	VSI	HSI	CF (g/cm^3)
MBM0	19.88 ± 0.26	5.07 ± 0.05	69.88 ± 0.37	5.05 ± 0.03	6.64 ± 0.11	1.11 ± 0.08^{bc}	1.62 ± 0.00
MBM0SH	19.45 ± 0.12	5.28 ± 0.10	70.06 ± 0.57	5.06 ± 0.05	6.76 ± 0.03	1.25 ± 0.03^{c}	1.60 ± 0.04
MBM25	18.98 ± 0.41	4.63 ± 0.19	69.83 ± 0.20	5.18 ± 0.01	7.40 ± 0.66	0.88 ± 0.07^{a}	1.53 ± 0.02
MBM25SH	19.21 ± 0.46	5.05 ± 0.04	69.62 ± 0.73	5.11 ± 0.03	6.61 ± 0.27	1.15 ± 0.02^{bc}	1.52 ± 0.02
MBM50	18.60 ± 0.32	4.15 ± 0.04	69.98 ± 0.52	5.19 ± 0.05	7.77 ± 0.13	0.95 ± 0.01^{ab}	1.45 ± 0.03
MBM50SH	19.20 ± 0.21	4.79 ± 0.10	69.86 ± 0.18	5.11 ± 0.01	8.02 ± 0.34	0.86 ± 0.05^{a}	1.61 ± 0.10
Means of main effects of dietary MBM							
0	19.67	5.18^{C}	69.97	5.05^{A}	6.70^{A}	1.18	1.61
25	19.10	4.84^{B}	69.73	5.14^{B}	7.01^{A}	1.01	1.53
50	18.83	4.35^{A}	69.88	5.15^{B}	7.90^{B}	0.90	1.53
Means of main effects of dietary SH supplementation							
0	19.15	4.62^{X}	69.83	5.14	7.27	0.98	1.53
45	19.24	4.96^{Y}	69.89	5.10	7.13	1.08	1.58
Two-way ANOVA: P values							
MBM	NS	<0.05	NS	<0.05	<0.05	<0.05	NS

SH	NS	<0.05	NS	NS	NS	<0.05	NS
MBM x SH	NS	NS	NS	NS	NS	<0.05	NS

Values are means of 3 replicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences (P < 0.05) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate significant differences (P < 0.05) among means of the main effects of MBM level. Means with different uppercase alphabets (X, Y) within a column indicate the significant difference (P < 0.05) among means of the main effects of SH supplementation. VSI Viscera-somatic index; HSI Hepatosomatic index; CF Condition factor.

Table 25. Fatty acid composition (% total fatty acids) in the muscle of giant trevally fed the test diets.

Diets	C14:0	C16:0	C17:0	C18: 0	SFA	C16:1n	C18:1n-9	C20:1n	C22:1n	MUFA
MBM0	2.38 ± 0.05	19.42 ± 0.16^{b}	2.38 ± 0.05	11.12 ± 0.62	35.29 ± 0.84^{b}	3.57 ± 0.08	21.04 ± 0.83^{b}	2.02 ± 0.11	1.19 ± 0.03	27.81 ± 0.89^{b}
MBM0SH	2.41 ± 0.03	18.90 ± 0.27^{b}	2.41 ± 0.03	11.26 ± 0.28	34.99 ± 0.29^{b}	3.23 ± 0.43	21.32 ± 0.26^{b}	2.17 ± 0.19	1.21 ± 0.01	27.93 ± 0.45^{b}
MBM25	1.47 ± 0.37	19.47 ± 0.35^{b}	1.47 ± 0.37	11.02 ± 0.63	33.56 ± 1.22^{b}	3.31 ± 0.00	23.14 ± 0.62^{bc}	1.95 ± 0.10	1.10 ± 0.00	29.62 ± 0.61^{b}
MBM25SH	1.88 ± 0.39	14.29 ± 0.49^{a}	1.50 ± 0.36	9.39 ± 0.32	27.25 ± 1.23^{a}	2.26 ± 0.03	16.55 ± 0.51^{a}	1.99 ± 0.05	1.13 ± 0.01	22.06 ± 0.26^{a}
MBM50	1.12 ± 0.02	18.37 ± 0.91^{b}	1.12 ± 0.02	11.23 ± 0.52	31.85 ± 0.42^{b}	3.00 ± 0.39	24.73 ± 0.70^{c}	1.65 ± 0.19	1.12 ± 0.02	30.51 ± 1.14^{b}
MBM50SH	1.51 ± 0.35	18.27 ± 0.28^{b}	1.92 ± 0.42	11.41 ± 0.35	33.12 ± 0.08^{b}	2.70 ± 0.49	23.26 ± 1.10^{bc}	1.87 ± 0.06	1.14 ± 0.04	28.97 ± 1.48^{b}
Means of main effects of dietary MBM										
0	2.40^{B}	19.16	2.40^{B}	11.19	35.14	3.40	21.18	2.10	1.20^{B}	27.87
25	1.68^{A}	16.88	1.48^{A}	10.21	30.40	2.78	19.85	1.97	1.12^{A}	25.84
50	1.32^{A}	18.32	1.52^{A}	11.32	32.48	2.85	23.99	1.76	1.14^{A}	29.74

Means of main effects of dietary SH supplementation										
0	1.66	19.09	1.66	11.12	33.57	3.29^{Y}	22.97	1.87	1.14	29.31
45	1.93	17.16	1.95	10.69	31.79	2.73^{X}	20.37	2.01	1.16	26.32
Two-way ANOVA: P values										
MBM	<0.05	<0.05	<0.05	NS	<0.05	NS	<0.05	NS	<0.05	<0.05
SH	NS	<0.05	NS	NS	<0.05	<0.05	<0.05	NS	NS	<0.05
MBM x SH	NS	<0.05	NS	NS	<0.05	NS	<0.05	NS	NS	<0.05

Values are means of 3 replicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate significant differences ($P < 0.05$) among means of the main effects of MBM level. Means with different uppercase alphabets (X, Y) within a column indicate the significant difference ($P < 0.05$) among means of the main effects of SH supplementation.

Table 26. Fatty acid composition (% total fatty acids) in the muscle of giant trevally fed the test diets (cont.).

Diets	C18:2n-6	C18:3n-3	C20:4n-6	C20:5n-3	C22:6n-3	PUFA	n-3PUFA	n-6PUFA	n-3/n-6	n-3HUFA
MBM0	6.36 ± 0.52	2.38 ± 0.05	1.35 ± 0.07	5.14 ± 0.29	18.26 ± 0.76	33.48 ± 1.24	25.10 ± 0.58	7.71 ± 0.59	3.28 ± 0.17	23.40 ± 0.62
MBM0SH	6.44 ± 0.42	2.00 ± 0.39	1.33 ± 0.05	5.63 ± 0.36	18.90 ± 0.27	34.30 ± 1.29	25.86 ± 0.65	7.77 ± 0.42	3.34 ± 0.14	24.53 ± 0.61
MBM25	6.24 ± 0.36	1.47 ± 0.37	1.18 ± 0.10	3.67 ± 0.37	17.27 ± 0.37	29.95 ± 0.98	22.21 ± 0.56	7.45 ± 0.34	3.00 ± 0.18	21.03 ± 0.65
MBM25SH	6.02 ± 0.46	1.13 ± 0.01	1.32 ± 0.10	4.69 ± 0.45	18.03 ± 0.45	31.43 ± 0.97	24.25 ± 1.26	7.38 ± 0.34	3.32 ± 0.32	22.92 ± 1.30
MBM50	8.24 ± 0.35	1.12 ± 0.02	0.56 ± 0.12	3.52 ± 0.12	14.99 ± 0.37	28.43 ± 0.54	19.06 ± 0.19	8.80 ± 0.32	2.17 ± 0.06	18.51 ± 0.26
MBM50SH	6.87 ± 0.24	1.14 ± 0.04	0.61 ± 0.09	3.69 ± 0.09	17.95 ± 0.90	30.27 ± 1.19	22.25 ± 0.91	7.48 ± 0.31	2.98 ± 0.05	21.64 ± 0.87

Means of main effects of dietary MBM										
0	6.40^{A}	2.19^{B}	1.34^{B}	5.38^{B}	18.58^{B}	33.89^{B}	25.48^{C}	7.74	3.31^{B}	23.96^{B}
25	6.14^{A}	1.30^{A}	1.25^{B}	4.18^{A}	17.65^{AB}	30.69^{A}	23.23^{B}	7.41	3.16^{B}	21.97^{A}
50	7.55^{B}	1.14^{A}	0.59^{A}	3.61^{A}	16.47^{A}	29.35^{A}	20.66^{A}	8.14	2.57^{A}	20.07^{A}
Means of main effects of dietary SH supplementation										
0	6.95	1.66	1.03	4.11^{X}	16.84^{X}	30.62	22.13^{X}	7.99	2.82^{X}	20.97^{X}
45	6.44	1.43	1.09	4.67^{Y}	18.29^{Y}	32.00	24.12^{Y}	7.54	3.21^{Y}	23.03^{Y}
Two-way ANOVA: P values										
MBM	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	NS	<0.05	<0.05
SH	NS	NS	NS	<0.05	<0.05	NS	<0.05	NS	<0.05	<0.05
MBM x SH	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are means of 3 replicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate significant differences ($P < 0.05$) among means of the main effects of MBM level. Means with different uppercase alphabets (X, Y) within a column indicate the significant difference ($P < 0.05$) among means of the main effects of SH supplementation.

4.3.3. Histological examination

Histological examination showed normal hepatocytes in all feeding groups (Figure 15). The MBM and SH interaction significantly affected the number of goblet cells (GCs) in the distal intestine of giant trevally ($P < 0.05$). Fish fed MBM25 and MBM50 had the lowest GCs compared to other dietary groups. The highest GCs were observed in fish fed the MBM0, MBMSH and MBM25SH diets. At each replacement level, the SH supplementation significantly increased GCs compared to those fed MBM without SH supplementation (Figure 16, Figure 17).

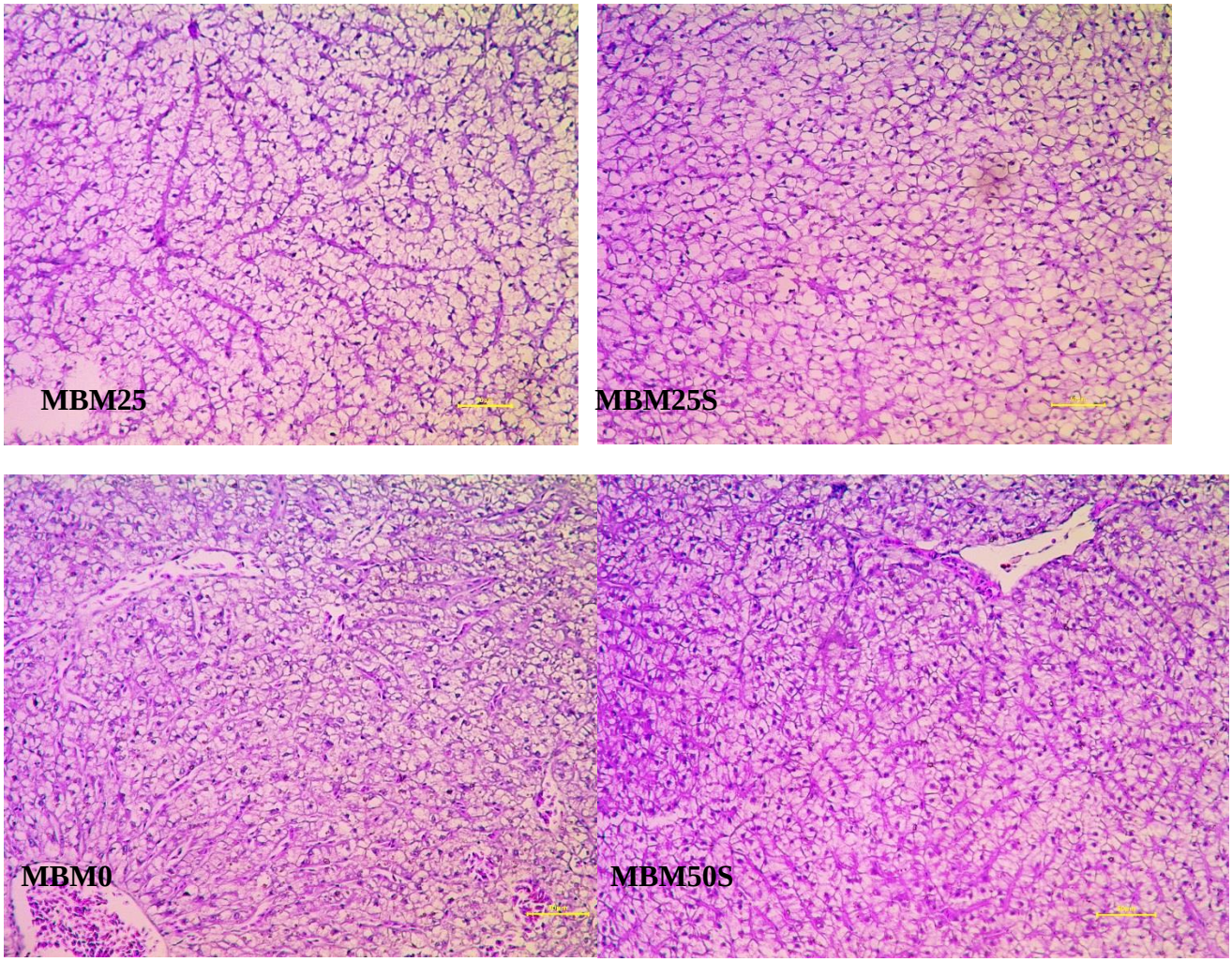


Figure 15. Liver histopathology of juvenile giant trevally fed test feeds.

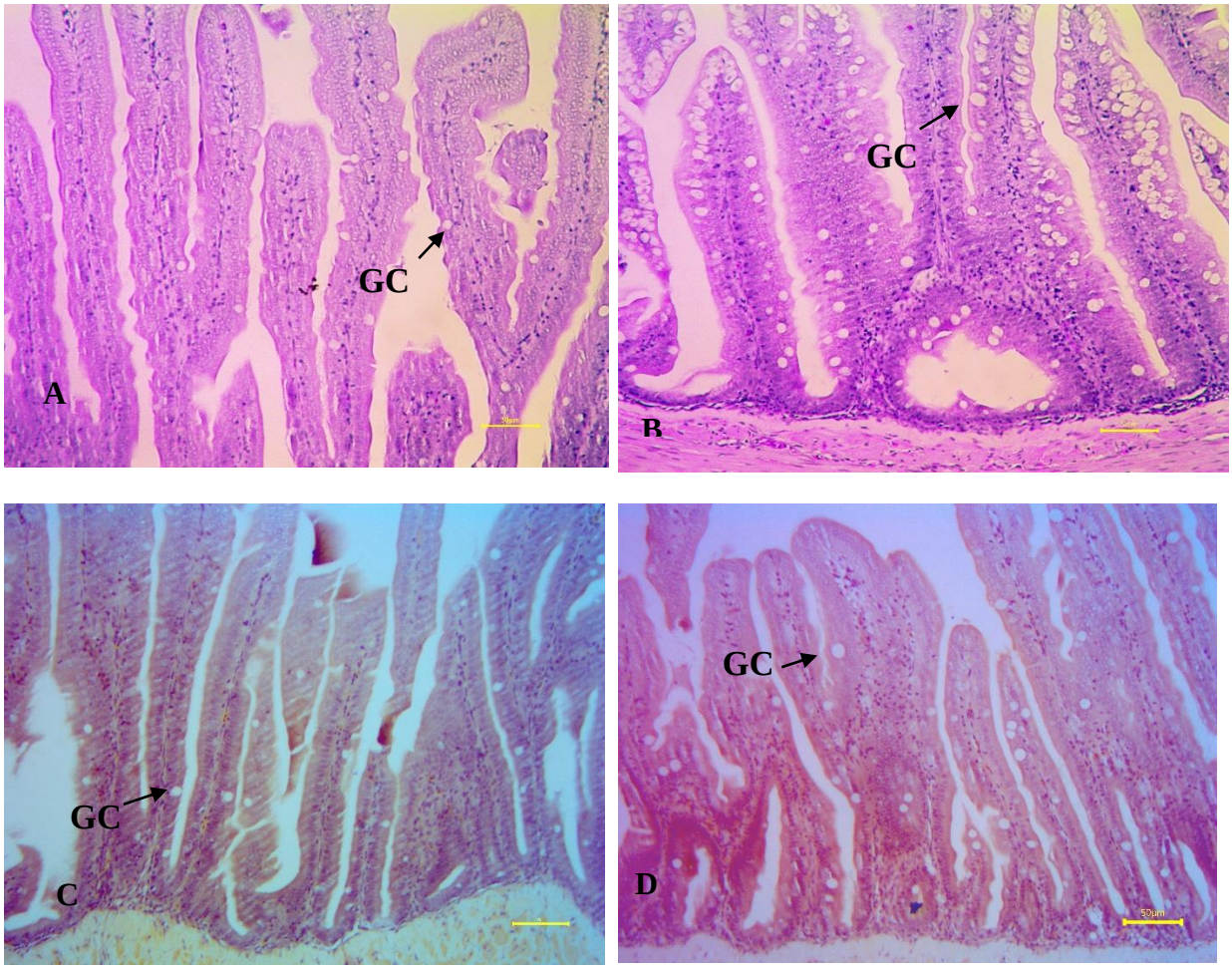


Figure 16. Representative micrographs of distal intestine of giant trevally after 8 weeks of being fed with MBM25, MBM25SH, MBM50 and MBM50SH. The intestine from MBM25 and MBM50 shows reduced goblet cells (A, C) while increased number of goblet cells were observed in MBM25SH and MBM50SH fed fish (B, D).

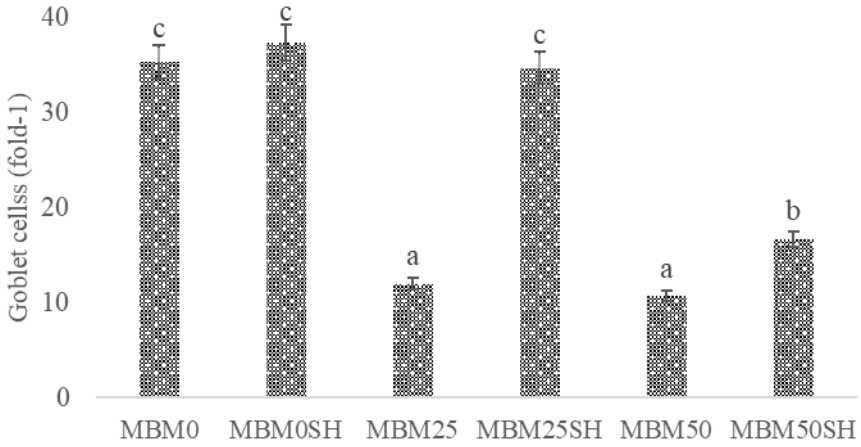


Figure 17. Representative micrographs of distal intestine of giant trevally. Different lowercase alphabets (a, b, c) indicate the significant differences ($P < 0.05$) among treatments.

4.3.4. Haematological parameters

SH supplementation did not influence the blood biochemical parameters, whereas dietary MBM significantly affected (P<0.05) total protein (TP), aspartate (AST) and alanine aminotransferase (ALT). Fish fed 311 g/kg MBM had significantly lower TP than those fed the control diet, while the AST was significantly reduced as increasing dietary MBM more than 155.5 g/kg diet. There was no difference on TP and AST of the fish fed the control and 155.5 g/kg MBM diets. ALT was affected by the interaction between dietary MBM level and SH supplementation, as lower ALT was seen in fish fed MBM50 than other remaining diets (Table 27).

Table 27. Blood/serum biochemical parameters of giant trevally fed test diets.

Diets	GLU	CHO	TGA	TP	AST	ALT
MBM0	5.44 ± 0.13	3.54 ± 0.07	3.53 ± 0.17	35.99 ± 0.92	42.23 ± 0.99	24.77 ± 0.31^{b}
MBM0SH	5.47 ± 0.10	3.21 ± 0.12	3.77 ± 0.22	36.79 ± 0.32	41.44 ± 1.23	24.32 ± 0.52^{b}
MBM25	5.51 ± 0.32	3.41 ± 0.14	3.77 ± 0.46	34.63 ± 0.76	39.14 ± 0.56	24.44 ± 0.45^{b}
MBM25SH	5.29 ± 0.11	3.43 ± 0.18	3.60 ± 0.26	34.65 ± 0.10	40.60 ± 0.49	24.24 ± 0.45^{b}
MBM50	5.41 ± 0.03	3.90 ± 0.26	3.42 ± 0.21	33.68 ± 0.96	35.93 ± 0.88	20.33 ± 0.54^{a}
MBM50SH	5.42 ± 0.30	3.62 ± 0.23	3.51 ± 0.03	33.75 ± 0.84	38.01 ± 0.42	22.56 ± 0.76^{b}
Means of main effects of dietary MBM						
0	5.46	3.38	3.65	36.39^{B}	41.83^{B}	24.55
25	5.40	3.42	3.69	34.64^{AB}	39.87^{B}	24.34
50	5.41	3.76	3.47	33.72^{A}	36.97^{A}	21.44
Means of main effects of dietary SH supplementation						
0	5.45	3.62	3.58	34.77	39.10	23.18
45	5.39	3.42	3.63	35.06	40.02	23.70
Two-way ANOVA: P values						
MBM	NS	NS	NS	<0.05	<0.05	<0.05
SH	NS	NS	NS	NS	NS	NS
MBM x SH	NS	NS	NS	NS	NS	<0.05

Values are means of 3 replicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significant differences ($P < 0.05$) among treatments. Means with different uppercase alphabets (A, B, C) within a column indicate significant differences ($P < 0.05$) among means of the main effects of MBM level. Means with different uppercase alphabets (X, Y) within a column indicate the significant difference ($P < 0.05$) among means of the main effects of SH supplementation. GLU: glucose; CHO: cholesterol; TGA: triglyceride; TP: total protein; AST: aspartate transaminase; ALT: alanine aminotransferase.

CHAPTER 5. DISCUSSIONS

5.1. The effects of dietary protein and lipid levels in juvenile giant trevally

At all dietary lipid levels, the FBW and SGR of juvenile giant trevally improved with increasing dietary protein from 42% to 52% as observed in other carnivorous fish that require high levels of good quality protein for growth (McGoogan & Gatlin, 1999). Fish exhibited the highest growth performance when fed a diet containing 52% protein and 10% lipid with P:E ratio of 24.63 g/MJ, which is the most suited diet for the growth of juvenile giant trevally, similar to the reported diet (24.53 g/MJ) of juvenile blackspotted croaker *Nibea diacanthus* (Li et al., 2017), and Florida pompano (22,97-24,18 g/MJ) (Riche, 2009) but lower than red-spotted grouper (29.83-30.58 g/MJ) (Wang et al., 2016b; Wang et al., 2017), and Malabar grouper (28 g/MJ) (Tuan & Williams, 2007). Different fish species, P/E ratio and feed ingredients may contribute to the differences in the optimal P/E ratios (Ai et al., 2004). Increasing lipids from 10% to 18% in 52% protein diets contributed to a decrease in the growth performance of fish, suggesting that 18% lipid is in excess for giant trevally fed 52% protein. The slightly sparing protein by energy occurred in the 42% protein diets, where the SGR improved with increasing dietary lipids from 10% to 18%. In low protein diets, increased energy from non-protein sources is likely to spare protein allocation for growth and maintenance (Riche, 2009). Protein sparing by energy, sourced from lipids, has been reported in red-tailed catfish (Hung et al., 2017), bagrid catfish (Kim & Lee, 2005), swordtails (Ling et al., 2006), permit (Nguyen et al., 2018), gilthead seabream (Santinha et al., 1999) and red-spotted grouper (Wang et al., 2017).

Carnivorous fish can easily digest energy from lipids rather than carbohydrates. However excess dietary lipids could lead to a decrease in the growth performance of fish (Bonvini et al., 2015; Regost et al., 2001; Wang et al., 2017). Wang et al. (2017) reported that 12% lipid as an excess level in diets containing 52% protein for juvenile red-spotted grouper. Black sole *Solea solea* decreased growth at diets containing above 12% lipid was seen (Bonvini et al., 2015). Juvenile giant croaker *Nibea japonica* fed dietary lipids of more than 13% showed a significant decrease in growth (Han et al., 2014). The present study indicated that 18% was the excess lipid content for juvenile giant travelly fed 52% protein diets. The excess dietary lipid levels could inhibit the

synthesis of *de-novo* fatty acid, and decrease the ability to digest and absorb it (Sargent et al., 1989). Moreover, it may cause not only imbalance of digestible energy/crude protein ratio but also excessive fat composition in the body (Murai et al., 1985). Excess energy in high lipid diets can have a negative effect on feed consumption and growth by the reduction of feed intake including dietary nitrogen (Bonvini et al., 2015; Khan & Abidi, 2012). Reduced FI was recorded in giant croaker (Han et al., 2014), black sole (Bonvini et al., 2015), and Senegalese sole *Solea senegalensis* (Borges et al., 2009) fed excess dietary lipid levels. However, the FI and FCR in the present study were significantly ($P < 0.05$) affected by the dietary protein instead of dietary lipid. This was consistent with Du et al. (2005), who stated that FI is likely to be regulated by protein intake rather than energy intake. Sa et al. (2006) recorded that dietary protein did not affect protein retention and apparent digestibility coeficient of protein and energy for white sea bream *Diplodus sargus* juveniles, thus suggesting that fish may prefer protein source to lipids or carbohydrates as energy source. Reduced feed consumption with increasing dietary protein was reported in white sea bream (Sa et al., 2008), blackspotted croaker (Li et al., 2017), channel catfish *Ictalurus punctatus* (Gaylord & Gatlin, 2001) and black catfish *Rhamdia quelen* (Salhi et al., 2004). The fish may try to increase FI to compensate for the lower dietary protein, in order to satisfy its protein requirement (Coutinho et al., 2012; Li et al., 2017; Sa et al., 2008). Conversely, increasing protein from 42% to 52% in the present study contributed to increased FI but reduced FCR. This may be caused by poorer palatability of low protein diets. Reduced FCR with increased dietary protein demonstrated that juvenile giant trevally was able to consume high protein diets for tissue synthesis more efficiently than the low protein diets.

Increased whole body protein was the result of the increase in dietary protein and lipid levels, indicating high dietary protein improved the protein synthesis in the tissue, and high lipids provided more efficient utilization of dietary protein for protein synthesis, suggesting the protein-sparing effect of energy in action. An increase in whole body protein with improved dietary protein has been reported for hybrid grouper (*Epinephelus fuscoguttatus x Epinephelus lanceolatus)* (Wang et al., 2016a), orange-spotted grouper *Epinephelus coioides* (Luo et al., 2004), Putitor mahseer *Tor putitora*

(Islam & Tanaka, 2004), and Chu's croaker *Nibea coibor* (Huang et al., 2017). The whole-body lipid value was significantly influenced by the interaction between dietary protein and lipids as high protein and lipids resulted in high lipid accumulation. Fish fed 52% protein and 18% lipid resulted in the highest body fat composition. This is in agreement with studies that claimed an increase in body lipid was the result of increased dietary lipid (Alam et al., 2004; Alam et al., 2009a; Peres & Oliva-Teles, 1999) and protein (Li et al., 2017). However, excessive lipid accumulation can affect the health status of the animals (Lee & Kim, 2001; Mathis et al., 2003), due to the possible link to changes in lipogenic enzyme activates (Dias, 2000). Liver is the main organ for storing lipids in majority of fish species, whereas muscle tissues are used as the major site to deposit lipids (Sheridan, 1988). Higher lipid deposition in the liver can lead to negative repercussions on the health of animals, resulting in higher levels of oxidative stress (Mathis et al., 2003). Some previous findings claimed that high dietary lipids led to a high fat composition in hepatic tissue or high HSI (Gómez-Requeni et al., 2013; Liu et al., 2013b; Wang et al., 2005). However, increased dietary lipid was also recorded to cause increased body lipid but decreased HSI value (Du et al., 2005; Liu et al., 2014; Peres & Oliva-Teles, 1999). A similar trend was observed in the current study, when the HSI pattern significantly ($P<0.05$) decreased with the increase in lipids from 10% to 18%. Takeuchi and Watanabe (1979) recorded a reduction of the liver weight in fish fed excess n-3 essential fatty acids. Du et al. (2005) claimed that higher HSI may be initial indicators of EFA deficiency. Whereas Peres and Oliva-Teles (1999) suggested that the possible correlation between hepatic glycogen and dietary carbohydrate may contribute to an HSI decrease. Similarly, increased hepatic glycogen with an increase in dietary carbohydrate was published in some previous studies (Coutinho et al., 2012; Cowey et al., 2008). In the present study, the diet containing 10% lipids had higher carbohydrate levels than 18% lipids for each protein group. This may explain the decrease in HSI with increasing lipids from 10% to 18%.

VSI is an important index directly affecting fish yield (Wang et al., 2005). Increased dietary lipids enhanced the VSI of giant croaker (Han et al., 2014), cobia (Wang et al., 2005), Pacific bluefin tuna *Thunnus orientalis* (Biswas et al., 2009), catfish *Silurus*

*asotus (*Liu et al., 2013a*)*, red-spotted grouper (Wang et al., 2017) and yellow drum *Nibea albiflora* (Wang et al., 2018); suggesting that high dietary lipids seemed to cause a poor commercial production value (Han et al., 2014; Wang et al., 2005; Wang et al., 2017). However, no significant effects of dietary lipids and protein alone or their interaction on the VSI were recorded in white seabream (Sa et al., 2006), blue gourami *Trichogaster trichopterus* (Mohanta et al., 2013), and tongue sole *Cynoglossus semilaevis (*Liu et al., 2013b*)*, as observed in the current study. CF is a crude indicator measuring the level of energy reserves, fish health (Goede & Barton, 1990) and the nutritional status of fish (Chatzifotis et al., 2010). Wang et al. (2017) found that dietary protein and its combination with lipids exhibited effects on the CF of red spotted grouper. The positive relationship between CF and growth performance was achieved in juvenile red spotted grouper (Wang et al., 2017) and turbot at medium (59.1 ± 0.24 g) and large size (209.1 ± 0.21 g) (Liu et al., 2014). A lower CF was observed in singhi *Heteropneustes fossilis* fed other dietary P/E ratios than optimum P/E ratio; indicating that fish was not comfortable with the excess levels of dietary protein and lipids (Khan & Abidi, 2012). In this study, the CF was not affected by dietary protein, lipids and their interaction, as a similar trend was reported for meagre *Argyrosomus regius* (Chatzifotis et al., 2010; Piccolo et al., 2008), turbot at small size (4.5 ± 0.01 g) (Liu et al., 2014), tongue sole (Liu et al., 2013b), hydrid grouper (*Epinephelus fuscoguttatus* ♀ x *E. lanceolatus* ♂) (Jiang et al., 2016), and blackspotted croaker (Li et al., 2017).

The diet of 52% protein and 10% lipid resulted in the highest isoleucine, lysine, methionine and threonine levels. Fish fed 52% protein diets achieved higher leucine, valine, arginine, alanine, aspartic acid, glutamine and serine levels than lower dietary protein; whereas 10% lipids contributed to increased levels of leucine, valine and arginine, alanine and glutamine in the whole body. These amino acids are materials for protein synthesis; they also serve as biological metabolites contributing to fish growth and immune improvement (Wang et al., 2021; Zhao et al., 2020). Leucine may improve muscle growth in fish by regulating IgF mRNA expression, muscle growth-related genes and protein synthesis-related signaling pathways (Zhao et al., 2020). Valine enhances fish's digestion by increasing absorbance capacity and balancing the

microflora system in the intestine (Ahmad et al., 2021). Arginine influences nutrient metabolism and improved disease resistance in fish (Wang et al., 2021). Alanine and glutamine are stimulatory amino acids (Wood & Azócar, 2013); alanine contributes to gluconeogenesis, while glutamine transfers carbons to the liver and kidney (Taziki et al., 2022). Alanine and glutamine stimulate the appetite of fish (Taziki et al., 2022). Serine can increase fish's survival upon bacterial infection by synthesing glutathione, which reduces excessive immune response (Yang et al., 2021). Muscle amino acid profile may be influenced by the quality of intake nutrition, for example protein content (Gunasekera et al., 1997). Li et al. (2017) claimed that the total EAAs and NEAAs in the whole body of blackspotted croaker increased when increasing protein from 42% to 52%. On the contrary, maximized EAAs in the whole body of Chu's croaker was achieved at 44% protein diets compared with dietary protein levels of 36%, 40%, 48% and 52% (Huang et al., 2017). Increased dietary protein may result in increased amino acid in whole body. However providing excess protein possibly leads to catabolism of excess amino acid into energy (Cuzon & Guillaume, 1997).

5.2. The effects of the supplementation of fish protein hydrolysate in juvenile giant trevally

Improved growth and reduced FCR was the result when giant trevally were fed 30 to 60 g/kg TH or SH, replacing 7% to 13% of the fishmeal, as seen in barramundi (Siddik et al., 2018b). Similarly, snubnose pompano (Pham et al., 2022), European seabass (Kotzamanis et al., 2007), Atlantic salmon (Hevroy et al., 2005) showed enhanced growth when fed dietary FPH inclusions of 60 g/kg, 100 g/kg and 92-121 g/kg FPH, respectively. The beneficial effects of dietary FPH on growth can be associated with the free amino acids and low molecular weight peptides, which are more likely to be absorbed and enhance nutrient uptake (Ospina-Salazar et al., 2016). Short-chain peptides in FPH improve feed palatability, and feed utilization in fish by reducing gluconeogenesis (Siddik et al., 2018b; Siddik et al., 2021a). Moreover, high antioxidant peptides in FPH may protect fatty acids from peroxidation, improving the growth and health of cultured animals (Siddik et al., 2018b). However, excessive dietary FPH levels can depress the growth of various fish species as FBW and SGR of giant trevally started to decline when fed diets containing 90 g/kg FPH. A similarly

reduced growth was observed in Japanese flounder fed 67 g/kg or higher dietary FPH levels (Zheng et al., 2014), snubnose pompano fed more than 60 g/kg tuna viscera hydrolysate (Pham et al., 2022) and turbot fed 124 g/kg FPH (Xu et al., 2016). Especially, the significant increase in dietary FPH inclusions of up to 589 g/kg not only deleteriously affected the growth, feed utilisation and digestibility but also increased liver injury in barramundi (Siddik et al., 2018a). The reduced growth can be linked to the saturation in the peptide transport mechanism (Ospina-Salazar et al., 2016) and unbalanced amino acids in the intestine (Carvalho et al., 2004) because of the excess of low-molecular-weight peptides and free amino acids. In addition, a non-optimal level of free amino acids influences the nutrient absorption time, affecting the utilisation of amino acids (Hevroy et al., 2005).

On the contrary, the appropriate quantities of shorter peptides in the FPH may perform as a chemo-attractant thus improving the palatability and finally the growth (Hevroy et al., 2005; Refstie et al., 2004; Siddik et al., 2018b; Xu et al., 2016). Kolkovski et al. (2000) found that krill hydrolysate-coated diets at the 5% level can increase the growth and feed consumption in yellow perch *Perca flavescens* and lake whitefish *Coregonus clupeaformis*. The authors postulated that the olfactory compounds of the krill hydrolysate-coated diets leached into the water, enhancing the taste and the palatability that resulted in attracting the fish to forage (Kolkovski et al., 2000). Though, palatability can be the main reason to elevate the feed ingestion and growth of fish fed dietary FPH (Hevroy et al., 2005; Refstie et al., 2004), the FPH did not impact the feed intake of giant trevally in the present study, as seen in snubnose pompano (Pham et al., 2022), Atlantic cod (Aksnes et al., 2006a), rainbow trout (Aksnes et al., 2006b) and red seabream (Bui et al., 2014) indicating palatability may not be the only factor contributing towards the growth. Growth improvement due to FPH supplement can also be explained by the quick assimilation and effective absorbance of low molecular weight peptides and free amino acids in the FPH through intestine membrane (Aksnes et al., 2006a). In this study, fish fed TH60 and SH60 had significantly lower VSI than those fed the control, SH30 and TH30 diets. This is in contrast to observations reported in red snapper (Khosravi et al., 2015b), barramundi (Siddik et al., 2018a) and

snubnose pompano (Pham et al., 2022), in which dietary FPH supplementation had no effects on VSI. This distinction is unclear and needs to be further investigated.

FPH supplementation showed no effect on whole-body composition of Atlantic salmon (Hevroy et al., 2005), turbot (Oliva-Teles et al., 1999), red seabream (Bui et al., 2014), barramundi (Siddik et al., 2018a) and snubnose pompano (Pham et al., 2022), as seen in giant trevally except for whole-body lipid content. The whole-body lipid elevated in giant trevally fed TH inclusions of 90 g/kg, while dietary SH had no influence. On the other hand, the whole-body lipid of Japanese flounder elevated with increasing FPH inclusions from 23 to 45 g/kg and then declined with further increased FPH from 67 to 90 g/kg (Zheng et al., 2014). Siddaiah et al. (2022) also reported increased lipids in striped murrel *Chana striata* fed diet containing 100g/kg FPH, while higher dietary FPH supplementation of 150 g/kg resulted in decreased lipid levels. A similar trend was reported in turbot (Wei et al., 2016; Xu et al., 2016) and pike silverside *Chirostoma estor* (Ospina-Salazar et al., 2016) as whole-body lipid levels reduced at certain dietary FPH levels (Espe et al., 2012; Xu et al., 2016; Zheng et al., 2014). High dietary FPH may decrease fatty acid synthesis and contribute to lipid oxidation and energy expenditure, thereby reducing lipid deposition (Bjørndal et al., 2013; Liaset et al., 2009; Xu et al., 2016). The increased whole-body lipid in giant trevally fed TH90 may be associated with the increased lipid droplets in hepatocytes of fish fed this diet (Figure 14).

The liver is a crucial digestive organ, reflecting the nutritional impacts of food (Rašković Božidar et al., 2011). Hepatocyte changes due to excessive dietary FPH inclusions were reported in barramundi (Siddik et al., 2018a; Siddik et al., 2018b). In the present study, elevated lipid droplets in liver was recorded in fish fed diet containing 90 g/kg TH in exchange of 20% fishmeal. Conversely, normal cells were observed in fish fed SH30, SH60, SH90, TH30, TH60 and fishmeal-based diets, demonstrating better health. Increased lipid accumulation was recorded in barramundi fed 122 g/kg FPH in diets replacing 20% of fishmeal (Siddik et al., 2018b). Siddik et al. (2018a) found that 589 g/kg FPH and 606 g/kg FPH fermented hydrolysate caused liver damage in barramundi, including increased lipid droplet, vacuole degeneration, necrotic loci and necrosis. Excessive dietary FPH inclusions may boost lipid

peroxidation, thereby causing liver damage (Siddik et al., 2018a). Lipid accumulation in liver cells is considered as an indicator of the liver's metabolic failure (Caballero et al., 1999). However, no hepatic alteration was reported in the snubnose pompano fed dietary FPH levels ranging from 0 to 120 g/kg (Pham et al., 2022). The plasma AST and ALT are biomarkers of hepatic injury, where their increased values may cause hepatic cellular damages (Chaklader et al., 2020a; Siddik et al., 2018b). In the present study, the FPH did not alter AST and ALT, indicating that present FPH inclusions did not cause liver damage in giant trevally. However, lipid droplet deposition in hepatocytes of fish fed TH90 was a symptom of hepatic steatosis and may be a warning sign for the onset of liver damage.

Haematological parameters are normally used to evaluate fish health, nutritional status, metabolic activity and immunological response (Siddik et al., 2018a; Siwicki et al., 1994; Wiegertjes et al., 1996). In the present study, the FPH did not impact haematological parameters of giant trevally, except for total protein and blood cholesterol. Bui et al. (2014) also reported no change in haematological indices among red seabream fed diets supplemented with and without FPH. Pompano fed diets containing various FPH inclusions did not show any differences in blood indices except for the lowering of total protein in fish fed 120 g/kg FPH than 60 g/kg (Pham et al., 2022). A higher blood protein, albumin or globulin may be considered as key to an active immunological system (Siwicki et al., 1994) and boost for innate immune response (Wiegertjes et al., 1996). The present study showed significantly higher serum protein in fish fed SH30, SH60, TH30, fishmeal-based diet compared to TH90, indicating that the supplementation of 90 g/kg TH in diet may negatively impact the innate immunity of fish. However, Siddaiah et al. (2022) showed a fluctuating trend of serum protein in striped murrel when increasing dietary FPH from 0 to 150 g/kg diet. Cholesterol is an essential metabolite used to evaluate the nutritional status of fish (Jia et al., 2018), and can be affected by protein sources in diets (Chaklader et al., 2020a). Chaklader et al. (2020a) stated that adding FPH to the diets reduces the adverse effects of high-poultry-based diets in barramundi, which was indicated by the lower serum cholesterol, ALT, glutamate dehydrogenase and total bilirubin. Lower cholesterol associated with FPH-contained diets was found in snubnose pompano (Pham et al.,

2021b), red seabream (Kader et al., 2010), as seen in the current study. In contrast, dietary FPH inclusions up to 122 g/kg did not affect the cholesterol level of barramundi (Siddik et al., 2018b). Differences in FPH sources and inclusion levels, species, fish size and experiment conditions may contribute to these inconsistent findings (Chatzifotis et al., 2010; Pham et al., 2022).

The effects of FPH on fatty acid composition were studied in turbot (Xu et al., 2016), barramundi (Chaklader et al., 2020a) and snubnose pompano (Pham et al., 2022) where increased FPH inclusion up to 124 g/kg resulted in the elevation of SFA but the reduction of PUFA in the turbot's muscle (Xu et al., 2016). Whereas SFA, MUFA and PUFA in the muscle increased when barramundi were fed poultry-meal-based diets added with appropriate FPH levels (Chaklader et al., 2020a). A similar result was recorded in snubnose pompano, wherein increased FPH inclusions resulted in enhanced whole-body PUFA, although no difference was found in MUFA (Pham et al., 2022). Increased PUFA is assumed to result from FPH supplementation (Chaklader et al., 2020a), which showed modulatory effects on lipid metabolism and fatty acid composition (Hosomi et al., 2011; Xu et al., 2016). High tissue fatty acids, particularly MUFA and PUFA, are beneficial for human health as they are shown to minimize neurological and cardiovascular disease risks (Michielsen et al., 2019). In the present study, despite lower ∑n-3 PUFA in fish fed TH30 compared to a fishmeal-based diet, the total SFA, MUFA and PUFA was not different among experimental diets. Excessive FPH levels negatively affected the contents of long-chain polyunsaturated fatty acids such as EPA and DHA of barramundi (Chaklader et al., 2020a). In contrast, EPA and DHA in the present study were unaffected by the dietary FPH, as observed in snubnose pompano (Pham et al., 2022).

5.3. The effect of shrimp hydrolysate on the efficacy of meat and bone meal diet in juvenile trevally

MBM has been used as a potential alternative protein source to fishmeal in diets of aquatic animals. However, the results also revealed negative effects in marine fish fed high MBM inclusion diets. Song et al (2016) reported reductions in growth and feed efficiency of turbot fed 342 g/kg MBM, while this threshold MBM levels in rainbow trout (Bureau et al., 2000; Esmaeili et al., 2017), large yellow croaker (Ai et al., 2006)

and Japanese flounder (Kikuchi et al., 1997) were 381.6, 434.4 and 360 g/kg diets. These findings were consistent with the present study wherein 25% and 50% of dietary fishmeal protein replaced by MBM protein without SH supplementation caused lower growth and feed intake, and higher FCR in giant trevally. Unbalanced amino acid profile and lower digestibility of MBM can contribute to the reduced growth and feed utilisation in fish (Ai et al., 2006). High levels of poorly digested ash in MBM may also result in low digestibility (Bureau et al., 1999). Robaina et al. (1997) showed that diets containing more than 12,5% ash had negative effects on protein digestibility. Therefore, dietary ash increased from 10% to 17,97% as increasing dietary MBM up to 311 g/kg diet could negatively affected nutrient digestibility, resulting in growth reduction of giant trevally fed high MBM diets in this study. Less palatability and the abundance of SFA and MUFA acids in MBM may be responsible for the reduction of feed intake, thereby decreasing the growth of fish (Turchini et al., 2009; Xue & Cui, 2001). The supplementation of essential nutrients, especially derived from marine by-product including FPH, may negate the adverse effects presented by feeding a diet of high non-fishmeal protein source (Kader et al., 2012; Khosravi et al., 2015c). Previous studies reported that the FPH effectively reduced the negative impacts on the growth and welfare of fish, which were caused by inclusion of plant meal (Costa et al., 2020; Gisbert et al., 2018; Khosravi et al., 2018) and poultry by-product meal (Chaklader et al., 2021; Pham et al., 2021b; Siddik et al., 2019). The reason for this could be high levels of free amino acid, available peptide fractions, digestibility, excellent viscosity (Saidi et al., 2014; Swanepoel & Goosen, 2018), palatability (Refstie et al., 2004), and bioactive properties (Costa et al., 2020) in FPH, which are suitable for intestinal assimilation, nutrient digestion and absorption in aquatic animals. In this study, giant trevally fed 311 g/kg MBM diet supplemented with SH had no differences on growth and feed efficiency from those fed the control diet, indicating that SH supplementation could be the pathway to increasing fishmeal replacement levels in giant trevally diets. However, the fish fed highest MBM inclusion diets also had significantly lower survival rate than those fed the control, regardless of SH supplementation. This was consistent with observations on ussuri catfish and large yellow croaker fed high MBM inclusion diets (Ai et al., 2006; Tang et al., 2018). In this study, no pathological signs

were observed in fish during the feeding period and the liver tissues did not show any histopathological lesions among treatments. However reduced survival rate in fish fed high MBM diets could be attributed to the imbalance of essential amino acids and deficient PUFA in high MBM diets. In this study, most essential amino acids, especially methionine and lysine, reduced as increasing dietary MBM levels (Table 16), which could prevent the liver metabolism and induce liver lesions, resulting in reduced growth and survival as reported in ussuri catfish (Tang et al., 2016).

Published literature has provided a mixed response about using dietary MBM as a fishmeal protein replacement on proximate composition. For example, no differences were observed in body composition of hybrid striped bass *Morone chrysops x Morone saxatilis* (Bharadwaj et al., 2002), gilthead seabream (Robaina et al., 1997) and rainbow trout (Bureau et al., 2000) fed up to 450, 280 and 240 g/kg MBM inclusion diets, respectively. Whereas, ussuri catfish showed reduced protein and lipid contents in the whole-body and muscles as increasing dietary MBM more than 200 g/kg diets (Tang et al., 2018). Ai et al. (2006) also reported the increased moisture and reduced lipid levels in the body of large yellow croaker fed more than 434.4 g/kg MBM diets. In the present study, dietary inclusion of MBM did affect ash and lipid levels in the muscles, although no change was observed in protein and moisture levels. Ash content increased while lipid decreased with increased dietary MBM levels, as seen in large yellow croaker (Ai et al., 2006) and ussuri catfish (Tang et al., 2018). High ash deposition in the muscle of giant trevally was directly related to high ash level in MBM based diets. Lower muscle lipid in fish fed MBM diets may be associated with low lipid digestibility of MBM diets and lipid metabolism in fish. Watanabe (1982) stated that the high SFA level in MBM reduced lipid digestibility in rainbow trout. Declined n-3 HUFA in fish with increased dietary MBM levels in the present study confirms the findings of Ai et al. (2006) that high MBM diets inhibit lipid metabolism. High dietary FPH can reduce fatty acid synthesis, but promote lipid oxidation and energy utilisation, leading to low lipid accumulation (Bjørndal et al., 2013; Liaset et al., 2009; Xu et al., 2016). However, low FPH levels caused an increase in body lipid as seen in Japanese flounder fed 45 g/kg FPH (Zheng et al., 2014) and rainbow trout fed 16-32% FPH inclusions. The present result found that 45 g/kg SH enhanced the

muscle lipid levels and the synthesis of HUFA (Table 6), which might compensate for lipid and unsaturated fatty acid deficiency caused by replacing 25% fishmeal with MBM, resulting in similar HSI between fish fed MBM25SH and fishmeal-based diet.

Hematological analysis showed lower total serum protein in fish fed diets replacing 50% fishmeal with MBM compared to fish fed non-MBM diets while there was no difference in serum glucose, cholesterol and triglyceride among any feeding treatments. Siwicki et al. (1994) stated that improved total protein links to stronger innate immunity of fish. High total protein and a low ratio of albumin/globulin indicate good health (Pham et al., 2021b; Siwicki et al., 1994). Declined total protein may impact the welfare of giant trevally fed diets substituting 50% fishmeal with MBM. Plasma ALT and AST reflect liver health; they leak into the blood at abnormal levels if the hepatic cells are impaired due to any stress (Ye et al., 2019). The use of dietary non-fishmeal protein resources has been reported to negatively affect the ALT and AST of fish (Chaklader et al., 2020b; Ye et al., 2019). Increased plasma ALT and AST were observed in hybrid grouper (*Epinephelus fuscoguttatus* ♀ *x Epinephelus lanceolatus* ♂) fed dietary animal protein blend replacing fishmeal levels at 20-80% and 80% respectively, leading to suppressed immune competence (Ye et al., 2019). Similarly, the serum AST in barramundi increased when they were fed poultry by-product, causing liver injury (Chaklader et al., 2020b). On the contrary, no changes in AST and ALT caused by poultry by-product inclusions in diets were recorded in barramundi (Chaklader et al., 2021; Siddik et al., 2019). In the present study, plasma AST and ALT even decreased in fish fed diets substituting MBM for 50% of fishmeal, indicating that replacing 50% fishmeal with MBM did not damage the hepatic cells in giant trevally. Besides, the histological observation showed no alteration in the livers of all fish fed any diets, additionally proving that replacing up to 50% fishmeal with MBM did not impair the liver of juvenile giant trevally.

The intestine is a crucial immune organ in fish. Improved intestinal health enhances the mucosal epithelium preventing microbial infections (Siddik et al., 2019). The intestine epithelium is significantly affected by nutrition intake and insufficient nutrition can change the intestine micromorphology such as fold height and goblet cell density (Siddik et al., 2018a). Goblet cells generate mucus that contains several

defensive substrates, influencing the innate immunity of the host species (Chaklader et al., 2021). The present result showed higher goblet cell density in the intestine of fish fed diets with SH supplementation than fish fed diets without SH (Figure 16, Figure 17), indicating that SH supplementation improved intestine health of giant trevally fed MBM replacing up to 50% fishmeal protein. The result was consistent with the finding in pompano, wherein adding 10% viscera hydrolysate protein increased the goblet cell number and fold heights of the intestine (Pham et al., 2022). Chaklader et al. (2020a) reported the supplementation of various hydrolysates with poultry by-product elevated the goblet cell number in barramundi and increased the goblet cell densities, enhancing the innate immunity against microorganisms (Siddik et al., 2018b). Hydrolysate supplementation also healed the intestine inflammation of Florida pompano fed high plant-based diets (Novriadi et al., 2017).

Fatty acids in the muscle of giant trevally were affected by substituted MBM levels and supplemented SH. Regardless of SH supplementation, fish fed dietary MBM replacing 50% fishmeal had the highest MUFA, as elevated fillet MUFA with increasing dietary MBM levels in rainbow trout (Esmaeili et al., 2017) and large yellow croaker (Ai et al., 2006). The fatty acid composition in muscle mirrors the dietary fatty acids, which involved the abundance of MUFA and SFA and the deficiency of PUFA and HUFA in high MBM based diets (Watanabe, 1982). Giant trevally fed MBM diets also showed poorer DHA, EPA, PUFA and n-3 HUFA than fish fed fishmeal-based diet, as seen in large yellow croaker (Ai et al., 2006), and rainbow trout (Esmaeili et al., 2017). Besides, the lack of PUFA and HUFA in dietary MBM, and increased oxidation may cause the reduction of PUFA and HUFA in fish fed dietary MBM (Esmaeili et al., 2017). However, lower muscle SFA was obtained in giant trevally fed dietary MBM, inconsistent with the finding in rainbow trout (Esmaeili et al., 2017), which may be due to the use of these fatty acids for energy production. Besides, the present result showed lower SFA and MUFA and higher DHA, EPA, PUFA, n-3 PUFA, n-3/n-6 HUFA and n-3HUFA in fish fed SH supplemented diets compared to fish fed SH non-supplemented diets. The result agreed with Pham et al. (2022), who stated that PUFA, n-3PUFA increases with increasing FPH substitution to fishmeal from 5% to 20%. Likewise, the significantly

elevated levels of PUFA, n-3HUFA, n-6HUFA, n-3/n-6HUFA in muscle were noticed in barramundi fed diets containing high poultry by-product level with FPH supplementation (Chaklader et al., 2020a). The change in PUFA and HUFA in fish fed FPH may be due to the modulatory effects of FPH on lipid and fatty acid metabolism-related genes, which were noted in turbot (Xu et al., 2016), barramundi (Chaklader et al., 2021), snubnose pompano (Pham et al., 2022) and mice (Bjørndal et al., 2013). Chaklader et al. (2021) reported that total fishmeal replacement by poultry by-product with the supplementation of FPH and black soldier fly *Hermetia illucens* larvae improved PUFA and MUFA in muscle.

5.4. The performance of giant trevally in relation to fish size

Analysing the growth performance of giant trevally fed fishmeal-based diets containing 52% protein and 10% lipid in the current research showed that smaller fish grew faster than larger ones (Table 28) similar to the finding of Akbulut et al. (2002), who recorded reduced specific growth with increasing body size of rainbow trout. The inverse relation between size and growth was also reported in several fish species including spotted wolffish *Anarhichas minor* (Imsland et al., 2006), North sea plaice *Pleuronectes platessa* (Rijnsdorp, 1993), turbot (Imsland et al., 1996), and halibut (Jonassen et al., 1999).

The variation in growth between fish sizes may be associated with social hierarchical establishment (Larsen et al., 2011). Social hierarchies are obvious in some fish populations, especially in carnivorous fish species, characterised by highly aggressive feeding behavior such as quicker food grabbing in the large fish than the smaller ones (MacLean et al., 2000; McCarthy et al., 1999). Therefore, size-grading fish allows all individuals to get a similar chance to forage for food and avoid cannibalism, thus reducing mortality (Kamstra, 1993). However, size separation may affect social structure, which is established by successive dyadic encounters between members of the population (Chase et al., 2003). Thus, individuals in a homogenous-sized population may face increased intra-specific interactions and conflicts when building a social hierarchy, thereby increasing energy expenditure and reducing growth (Baardvik & Jobling, 1990). This hypothesis was proved in eel *Anguilla anguilla* (Kamstra, 1993), Arctic charr *Salvelinus alpinus* (Baardvik & Jobling, 1990), turbot

(Strand & Oiestad, 1997; Sunde et al., 1998), and Atlantic halibut *Hippoglossus hippoglossus* (Stefánsson et al., 2000), where the size-graded group obtained lower growth than the ungraded group. Furthermore, larger fish are normally stronger and more aggressive, thus individuals in larger-sized fish population may experience intense social interaction and conflicts, leading to lower growth compared to smaller-sized fish ones, as seen in the present research. Moreover, in all my experiments, all treatments used the same fish number and tank size, meaning that the larger group had less space among themselves, requiring more energy for their interaction and competition.

Table 28. Growth performance, feed utilization and survival of giant trevally fed fishmeal-based diet containing P52:L10 diets in three experiments.

Diets	Initial size (g)	SGR (%/day)	FI (g/fish)	FCR	Survival (%)
P52:L10 (Exp.1)	2.12 ± 0.05	4.05 ± 0.01^{b}	20.36 ± 0.72^{a}	1.00 ± 0.04^{a}	95.56 ± 1.11
Control (Exp.2)	4.12 ± 0.03	2.86 ± 0.04^{a}	27.33 ± 0.88^{b}	1.67 ± 0.01^{b}	89.33 ± 2.67
MBM0 (Exp.3)	5.11 ± 0.04	2.78 ± 0.03^{a}	29.67 ± 0.67^{b}	1.56 ± 0.03^{b}	93.33 ± 1.33

Values are presented as the mean of triplicate groups ± SE. Different lowercase alphabets (a, b, c) in the same row indicate significant differences ($P < 0.05$) among treatments.

5.5. Beneficial effects of moderate FPH inclusion levels (30-60 g/kg) in the diets for juvenile giant trevally

The comparison of SGR between fish fed diets with and without 30-60 g/kg FPH showed that the supplementation of moderate inclusion levels of FPH in fishmeal-based or MBM-based diets improved the growth of juvenile giant trevally (Figure 18). Moreover, the current research confirmed that parameters indicating feed utilization, body composition, and biochemical and histological responses in fish fed diets with moderate FPH either equal or even better than those fed the same diets without FPH (Table 29). The availability of free amino acids and peptide fraction in FPH, produced during enzymatic hydrolysis, may stimulate feed utilization and growth of fish (Ospina-Salazar et al., 2016; Siddik et al., 2018b). High digestibility and palatability make FPH an excellent attractant, enhancing the utilization of low digestibility diets as

high MBM diets (Chotikachinda et al., 2013). Moreover, FPH supplementation provides essential amino acids (Siddik et al., 2019), which can improve the quality of fishmeal diets, and can compensate for limited essential amino acids in high MBM diets. Bioactive properties in FPH also enhance immune function and disease resistance (Khosravi et al., 2015a; Liang et al., 2006; Murray et al., 2003) . The positive effects of moderate inclusion levels of FPH on the growth, feed utilization and physiological response in the present research were also recorded in several other fish species including European seabass (Kotzamanis et al., 2007) and Atlantic salmon (Hevroy et al., 2005) fed fishmeal-based diets; barramundi (Siddik et al., 2019) and snubnose pompano (Pham et al., 2021b) fed high poultry by-product meal diets; Japanese flounder (Zheng et al., 2014) and turbot (Xu et al., 2017) fed high plant-based diets.

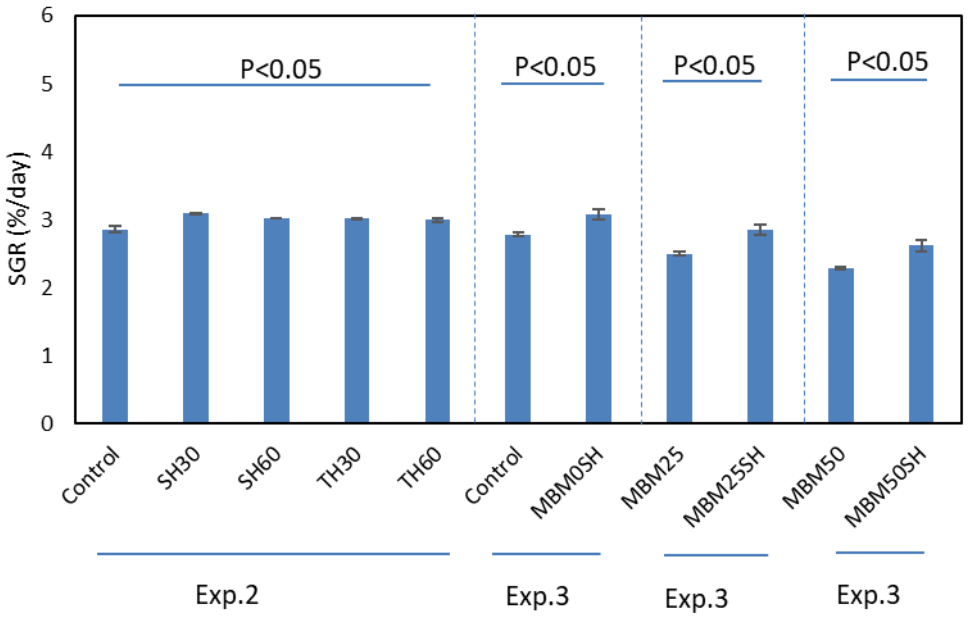


Figure 18. Specific growth rate (SGR) of juvenile giant trevally fed fishmeal-based diets and MBM based diets with and without FPH supplementation in experiment 2 and experiment 3. The bars with P<0.05 in the same experiment indicate the statistically significant.

Table 29. Growth performance, feed utilization, body proximate composition and physiological response of giant trevally fed diets with FPH supplementation.

Parameters	Experiment diets						
	SH30	SH60	TH30	TH60	MBM0SH	MBM25SH	MBM50SH
Growth indices							
FBW (g)	↑	↑	↑	↑	↑	↑	↑
SGR (%/day)	↑	↑	↑	↑	↑	↑	↑
Feed utilization							
FI (g/fish/day)	↔	↔	↔	↔	↑	↑	↑
FCR	↓	↓	↓	↓	↔	↔	↓
Body proximate composition							
Ash (%)	↔	↔	↔	↔	↔	↔	↔
Lipid (%)	↔	↔	↔	↑	↑	↑	↑
Protein (%)	↔	↔	↔	↔	↔	↔	↔
Biochemical indices							
TP (g/L)	↔	↔	↔	↓	↔	↔	↔
CHO (nmol/L)	↔	↔	↔	↔	↔	↔	↔
AST (µ/L)	↔	↔	↔	↔	↔	↔	↔
ALT (µ/L)	↔	↔	↔	↔	↔	↔	↑
Histological examination							
Goblet cell in gut					↔	↑	↑

Liver histopathology	↔	↔	↔	↔	↔	↔	↔

Increase ↑, decrease ↓, no change ↔ compared to diets with the same protein levels and sources but without FPH supplementation ($P<0.05$); FBW final body weight; SGR specific growth rate; FI feed intake; TP total protein; TGA tryglyceride; AST aspartate transaminase; ALT alanine aminotransferase.

5.6. A comparison of the growth and survival of juvenile giant trevally between existing commercial diets and the recommended diet from the current research

Table 30. Growth and survival of juvenile giant trevally fed test diets and those in commercial scale.

Treatment	Description	SGR (%/day)	Survival (%)
P52:L10	- The best treatment in experiment 1 - Reared in indoor 250L-tank, 30 ind./tank - Feeding 2 times/day - Initial weight: 2.12 ± 0.05 g/fish - Duration: 56 days	4.05 ± 0.01	95.56 ± 1.11
TH30	- The best treatments in experiment 2 - Reared in indoor 250L-tank, 30 ind./tank - Feeding 2 times/day - Initial weight: 4.12 ± 0.03 g/fish - Duration: 56 days	3.02 ± 0.01	86.67 ± 1.33
TH60	- The best treatments in experiment 2 - Reared in indoor 250L-tank, 30 ind./tank - Feeding 2 times/day - Initial weight: 4.12 ± 0.03 g/fish - Duration: 56 days	3.00 ± 0.03	89.33 ± 2.67
SH30	- The best treatments in experiment 2 - Reared in indoor 250L-tank, 30 ind./tank - Feeding 2 times/day	3.09 ± 0.01	90.67 ± 1.33

	- Initial weight: 4.12 ± 0.03 g/fish - Duration: 56 days		
SH60	- The best treatments in experiment 2 - Reared in indoor 250L-tank, 30 ind./tank - Feeding 2 times/day - Initial weight: 4.12 ± 0.03 g/fish - Duration: 56 days	3.02 ± 0.00	89.33 ± 2.67
MBM25SH	- The selected treatment in experiment 3 - Reared in indoor 250L-tank, 30 ind./tank - Feeding 2 times/day - Initial weight: 5.11 ± 0.04 g/fish - Duration: 56 days	2.85 ± 0.07	85.33 ± 1.33
Fish in commercial cultured tanks	- Reared in indoor 5m^3-tank; 800-1000 fish/tank - Feeding 4 times/day - Initial weight: 2.53 ± 0.09 g/fish - Duration: 60 days - Feed: G12 (INVER, Thailand; 55% protein, 8-10% lipid)	4.05 ± 0.01	95.22 ± 1.06
Fish in commercial cultured sea cages	- Reared in sea cage: 10m^3/cage; 250-300 fish/cage - Feeding 4 times/day - Initial weight: 2.67 ± 0.09 g/fish - Duration: 60 days - Feed: G12 (INVER, Thailand; 55% protein, 8-10% lipid)	4.59 ± 0.03	83.73 ± 2.77

The comparison between the performance of giant trevally fed diets, which were suggested to be suited for fish in the current research and those in commercial scales showed similar growth and survival between fish fed P52L10 in experiment 1 and fish commercially reared in tanks (Table 30). However, fish fed P52L10 obtained lower

growth and higher survival than fish commercially cultured in sea cages. Fish reared in sea cages face more challenges because of fluctuating climatic and natural environmental conditions and various wild pathogens, causing stress and low survival whereas these disadvantages are controlled to the advantage for fish in indoor tanks (Premachandra et al., 2017). Although the above challenges also inhibit the growth of fish, fish may adapt well to natural biological conditions in the sea where they can catch highly nutritional zooplankton and phytoplankton in sea cages besides the formulated pellets provided, thus boosting their growth. Besides, different densities also caused the variance in growth and survival, as reported in largemouth black bass *Micropterus salmoides* (Jia et al., 2022), turbot (Jia et al., 2016), Nile tilapia (Abdel-Hakim et al., 2001) and Atlantic salmon (Liu et al., 2017). Overall, the different culture environments and stocking densities between tank culture and cage farming will be overarching influence on the growth and survival of the farmed juvenile fish species (Jia et al., 2016; Premachandra et al., 2017).

The growth was lower in fish fed TH30, TH60, SH30, SH60, MBM25SH than those in commercial cultured systems (Table 30). Larger size in fish fed test diets may cause poorer growth than fish in commercial scales. Smaller fish can consume feed more efficiently than larger ones, enhancing their growth, as seen in rainbow trout (Akbulut et al., 2002). Besides, the difference in feeding frequency between fish fed test diets (2 times/day) and fish in commercial systems (4 times/day) may contribute to variance in growth. Wu et al. (2021) found increased growth with increasing feeding frequency from 1 to 4 times daily in juvenile grass carp *Ctenopharygodon idellus.* Similarly, higher growth was achieved in juvenile Chinese sucker *Myxocyprinus asiaticus* fed 3-4 meals/day than those fed 1-2 meals/day (Yu et al., 2013). Rainbow trout required 3 meals/day for their maximum growth (Ruohonen et al., 1998), as seen in golden pompano *Trachinotus ovatus* (Wu et al., 2015b), tambaqui *Colossoma macropomum* (Silva et al., 2007), and Asian seabass (Biswas et al., 2010). The growth of fish directly depends on feed intake, where insufficient feeding causes poor growth (Wu et al., 2015a). However, feed intake in each meal must be limited because of restricted stomach storage (Busti et al., 2020) and gastric evacuation time of fish species (Wu et al., 2015b). For example, golden pompano has a small stomach and a short intestine

tract, requiring a high feeding frequency to effectively ingest feed (Wu et al., 2015b). Dividing a big meal into small meals satisfying feed requirements and avoiding feed waste are necessary for some fish species (Busti et al., 2020), but others only require 1-2 meals/day for their maximum growth, as seen in rockfish (Lee, 2000), cuneate drum *Nibea miichthioides* (Wang et al., 2007), Japanese flounder (Kim et al., 2007), and snapper (Booth et al., 2008). In the present research, the lower growth of fish fed test diets than fish in commercial cultures seems to be partly caused by less feeding frequency. However, this hypothesis needs to be further investigated to determine optimum feeding frequency for juvenile giant trevally.

Conclusions

The current results have met all the four objectives of the research. First, the results determined the effects of different protein and lipid levels with relevant P/E ratios on growth, feed utilization, and body composition of juvenile giant trevally. 52% protein and 10% lipid diet improved the SGR and FCR of giant trevally. High dietary protein of 52% improved the protein synthesis in the whole-body fish. Increasing lipid levels from 10% to 18% along with 52% protein resulted in a decreased growth rate, indicating 18% lipid was in excess for giant trevally fed 52% protein. Increased whole-body lipid was also seen in fish fed 52% protein and 18% lipid diet. Finally, the juveniles fed 52% protein and 10% lipid diet resulted in improved non-essential and essential amino acids, including isoleucine, lysine, methionine and threonine levels. 52% protein led to higher leucine, valanine, arginine, alanine, aspartic acid, glutamine and serine levels, whereas 10% lipid contributed to increased levels of leucine, valanine and arginine, alanine and glutamine in the whole body. Therefore, the results suggested 52% protein and 10% lipid with a P/E ratio of 24.63 g/MJ in the diet was optimum for the growth, feed utilization and body composition of juvenile giant trevally.

Second, the results showed the effects of various inclusion levels and sources of fish protein hydrolysate as partial alternatives to fishmeal protein in juvenile giant trevally. SGR was improved, and FCR was reduced in fish fed 30-60 g/kg TH or SH whereas reduced growth was recorded in fish fed 90 g/kg TH or SH. Fish fed 90 g/kg TH increased whole-body lipid and lipid droplets in the hepatocytes of fish fed this diet,

being a sign of the commencement of liver damage. FPH supplementation did not affect total SFA, MUFA and PUFA, except for lower ∑n-3 PUFA in fish fed TH30 compared to a fishmeal-based diet. The results suggested that TH and SH could be incorporated into diets of giant trevally at 30-60 g/kg, replacing 7-13% fishmeal with enhanced growth and health benefits.

Finally, the present results indicated that FPH supplementation can improve the efficacy of MBM diet in juvenile giant trevally. The substitution of 25% and 50% fishmeal protein with MBM protein without SH supplementation resulted in lower growth and feed intake but higher FCR. Whereas fish fed dietary MBM replacing 50% fish meal with 45 g/kg SH supplementation achieved growth and feed efficiency as high as fish fed the control diet. Muscle lipid content decreased with increasing dietary MBM levels. Conversely, 45 g/kg supplementation enhanced muscle lipid and HUFA synthesis, resulting in similar HSI between fish fed MBM25SH and control diets. Replacing 50% fishmeal with MBM caused lower serum total protein, negatively affecting the welfare of fish fed this diet. However, the liver health of fish fed 50% MBM diets was unaffected as there was no change in plasma ALT, AST and histological observation. SH supplementation enhanced the intestine health of fish fed dietary MBM substituting up to 50% fishmeal as higher goblet cell density in fish intestine. Dietary MBM caused higher MUFA but lower DHA, EPA, PUFA and n-3 HUFA than the control. However SH supplementation resulted in lower SFA and MUFA and higher DHA, EPA, PUFA, n-3 PUFA, n-3/n-6 HUFA and n-3HUFA compared to fish fed SH non-supplemented diets. The results indicated that supplemented SH of 45 g/kg enhanced the efficacy of substituting MBM protein for 50% of fishmeal protein without compromising the growth of juvenile giant trevally.

Limitations

- Although some immunological parameters in haematology were evaluated, immunological enzymes and capacity of disease resistance of fish fed high MBM diets with FPH supplementation have not been investigated.
- Although the results demonstrated that supplementation of SH can improve the efficacy of MBM protein replacing up to 50% fishmeal protein in giant trevally,

experiments were carried out at a laboratory scale where all environmental parameters and contamination were easy to control at suitable ranges for fish. However, the results of the research have not been applied to open field trials in sea-cages where fish may face adverse factors including the largely vary fluctuation of environmental conditions and pathogen contamination issue.

Recommendations

Based on the results and limitations of the research, the following recommendations are made. The future research should investigate:

- The effects of dietary FPH supplementation on gut health, microbial characterization, immunological enzymes and disease resistance of giant trevally fed high meat and bone meal diets.
- There is a need to elaborately examine how FPH supplementation affects VSI of giant trevally.
- The substitution of MBM for 50% fishmeal protein with SH supplementation should be validated in sea-cage trials to correct the finding before using in the commercial culture.
- Further study should be considered whether FPH supplementation in high meat and bone meal diets could be successful on other fish species.
- There is a need to evaluate the combination of FPH and other alternative protein sources in the diets of giant trevally.

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