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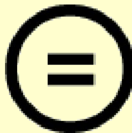

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A DISSERTATION

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Nutritional and immunological studies of red seabream (*Pagrus major*) and Pacific white shrimp (*Litopenaeus vannamei*) fed marine protein hydrolysates

G.L. Buddhi Eranga Gunathilaka

Department of Marine Life Science

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

2020.02.

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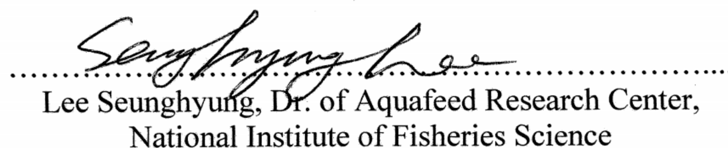
G.L. Buddhi Eranga Gunathilaka

(Supervised by professor Kyeong-Jun Lee)

A dissertation submitted in partial fulfilment of the requirement for the degree of
DOCTOR OF PHILOSOPHY

2020.02.

This dissertation has been examined and approved by,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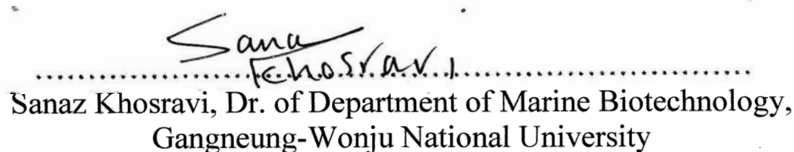
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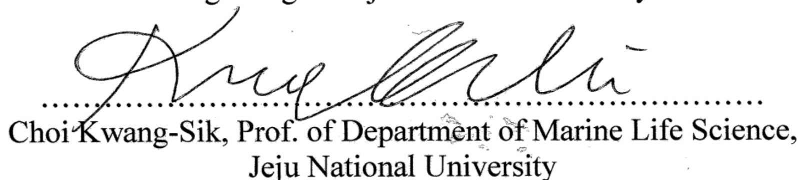
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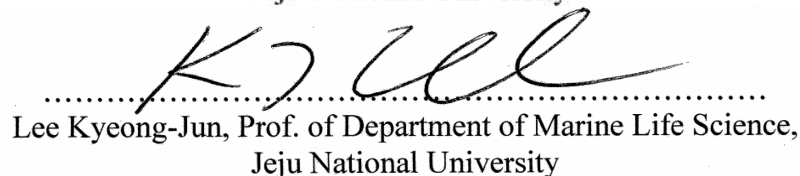
Jeju National University

Jung Sukgeun, Prof. of Department of Marine Life Science,
Jeju National University


Lee Kyeong-Jun, Prof. of Department of Marine Life Science,
Jeju National University

Department of Marine Life Science

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Abstract

The studies presented in this dissertation are aimed to evaluate supplementary effects of marine protein hydrolysates in low fish meal diets on growth performance, feed utilization, innate immunity, hematological parameters, diet digestibility, gut morphology and disease resistance of red seabream (*Pagrus major*) or Pacific white shrimp (*Litopenaeus vannamei*).

Chapter two was conducted to evaluate the effects of salmon protein hydrolysate (SalH), tilapia protein hydrolysate (TH) and tuna protein hydrolysate (TuH) supplemented low fish meal diet in red seabream diets. Two experimental diets were prepared as a high fish meal diet (HFM) to contain 36.8% fish meal (FM) and a low-FM diet (LFM) to contain 13.5% FM. Two other experimental diets were prepared by dietary inclusion of SalH and TH to LFM based diet at 5% inclusion level. One other diet was prepared by coating 2% TuH to LFM diet (designated as, SalH, TH and TuH respectively). Growth performance and feed utilization were significantly higher in fish fed HFM or protein hydrolysate diets compared to LFM group. Feed intake was significantly higher in fish fed HFM and TuH diets. Dry matter and protein digestibility were significantly higher in HFM, SalH and TH diets than the LFM diet. Innate immune responses of red sea bream were significantly improved by dietary supplementation of hydrolysates. Intestine morphological parameters were positively influenced by HFM, SalH, TH and TuH diets. Relative expression levels of liver IGF-1 mRNA was significantly higher in fish fed HFM, and protein hydrolysate diets. Survival rates were higher in HFM and hydrolysate groups than the LFM group after *Edwardsiella tarda* challenge. Therefore, these results indicate that reduced feed utilization and immune responses of red seabream by LFM diet can be recovered by supplementing marine protein hydrolysates.

In chapter three, supplemental effects of TH and/or shrimp hydrolysates (SH) were evaluated in red seabream diet. The HFM diet contained 37% FM and the LFM diet contained 18.5% FM. Three other experimental diets were prepared by dietary inclusion of TH, mixture of TH and SH or SH in low FM based diet at 5% inclusion level (designated as TH, TH/SH and SH). Growth and feed utilization of fish fed SH diet were significantly higher compared to HFM and LFM diet. Protein digestibility of fish fed SH was significant compared to the other dietary treatmentInnate immune responses and intestine morphometric parameters of red seabream were significantly improved by dietary supplementation of SH and/or TH. The highest survival rate was observed in the SH group against *Edwardsiella tarda*. Therefore, these results indicate that dietary SH and/or TH supplemented LFM diet, which contains 18.5% FM, can improve the performance of red seabreams better than a HFM diet which contains 37% FM.

Chapter four was conducted to examine the effects of SH or krill meal (KM) in a LFM diet for red seabream compared to a LFM and HFM diets. The HFM diet contained 40% FM and the LFM

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diet contained 25% FM. Two other experimental diets (SH and KM) were prepared by dietary inclusion of SH and KM in LFM diet at 5% inclusion levels in exchange of 12.5% FM from the LFM diet. Growth and feed utilization were significantly improved in fish fed HFM, KM and SH supplemented diet compared to LFM group. Interestingly, these parameters of fish fed SH diet were significantly higher than KM and HFM groups. Protein digestibility of SH diet was significantly higher than KM, HFM and LFM diets. DM digestibility of SH diet was comparable to HFM diet and significant than KM and LFM diets. Innate immune responses of fish fed SH diet were significantly enhanced compared to the LFM group. Hemoglobin level of fish fed SH and KM diets were significant to LFM group. Liver IGF-1 mRNA expression of SH diet fed fish group was comparable to HFM group. Therefore, Compared to KM, SH can be used to replace FM from red seabream diet down to 20% and fish performance can be maintained better than a 40% FM containing diet.

In chapter five, effects of TuH, SH, and DFS were examined on growth, feed utilization, intestinal morphology, innate immunity and disease resistance of Pacific white shrimp. A diet containing 4.49% of squid liver powder (SLP) was regarded as the control diet and three other diets were prepared by supplementing TuH, SH and DFS (designated as SLP, TuH, SH and DFS). Growth and feed utilization of shrimp were significantly improved by the SH diet. Innate immune responses of shrimp were enhanced by dietary SH supplementation. Shrimp fed the TuH, SH and DFS diets exhibited significantly higher lipid composition than SLP group. SH diet showed significantly higher dry matter digestibility than TuH, and DFS diets. Protein digestibility was significantly higher in SH diet compared to the other diets. Therefore, the performance of Pacific white shrimp can be effectively improved by dietary shrimp hydrolysates compared to SLP.

In chapter six, effects of different dietary levels of DFS, SH and TuH (2% or 4% DFS, 1% or 2% SH and 1.5% or 3% TuH) were evaluated in diets for Pacific white shrimp compared to a diet containing squid liver powder (designated as SLP, DFS-2, DFS-4, SH-1, SH-2, TuH-1.5 and TuH-3). Final body weight of shrimp fed SLP diet was significantly lower compared to other diets. Weight gain and protein efficiency ratio of shrimp were significantly improved by DFS-4, SH-1 and TuH-1.5 diets. Innate immune response parameters of shrimp were significantly improved by hydrolysate supplementation regardless of inclusion level. Hemolymph THC was significantly higher in SH-2, TuH-1.5 and TuH-3 groups compared to SLP group. Hyaline cell count was significantly lower in shrimp fed SLP diet. Protein digestibility was significantly higher in diets containing hydrolysates. Dry matter digestibility was significantly higher in DFS-2, SH-1, TuH-1.5 and TuH-3 diets compared to SLP diet. Disease resistance against *Vibrio harveyi* was significantly affected by DFS, SH and TuH diets. In conclusion, dietary supplementation of SH and TuH in these levels seems to improve growth and immunity of Pacific white shrimp among tested diets.

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Dedication

This thesis is dedicated to

everyone

who supported me

throughout the process and shares

their invaluable and irreplaceable

experience.

Without their support,

I could not have completed

this research work.

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Table of content

Chapter I........13

1. Protein sources used in aquafeed........13

   Fish meal........13

   Krill meal........14

   Squid liver powder........15

   Dried Fish soluble........16

2. Plant protein sources as an alternative to fish meal........16

3. Development of low fishmeal diets........17

4. Protein hydrolysates........18

5. Marine Protein hydrolysates........18

   Shrimp hydrolysates........19

   Tilapia hydrolysates........20

   Salmon hydrolysates........20

   Tuna hydrolysates........21

   Krill hydrolysates........22

6. Chapter justification........22

Chapter II........25

1. Introduction........25

2. Materials and Methods........26

   Experimental diets........26

   Fish and feeding trial........29

   Estimation of apparent digestibility coefficients........30

   Analyses........32

   Intestinal morphology........36

   Expression levels of liver IGF-I mRNA........37

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Challenge test 38

Statistical analysis 38

3. Results 38

4. Discussion 49

Chapter III 53

1. Introduction 53

2. Materials and Methods 54

Experimental diets 54

Fish and feeding trial 58

Sample collection 58

Estimation of apparent digestibility coefficients 59

Analyses 59

Intestinal morphology 60

Challenge test 61

Statistical analysis 61

3. Results 61

4. Discussion 69

Chapter IV 73

1. Introduction 73

2. Materials and Methods 75

Experimental diets 75

Feeding trial and sample collection 78

Sample collection and Analyses 78

Estimation of apparent digestibility coefficients 79

Expression levels of liver IGF-I mRNA 80

Statistical analysis 80

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

3. Results 80

4. Discussion 85

Chapter V 90

1. Introduction 90

2. Materials and Methods 92

Experimental diets 92

Shrimp and feeding trial 95

Sample collection 96

Estimation of apparent digestibility coefficients 96

Analyses 96

Challenge test 99

Statistical analysis 100

3. Results 100

4. Discussion 106

Chapter VI 109

1. Introduction 109

2. Materials and Methods 110

Experimental diets 110

Shrimp and feeding trial 114

Sample collection 114

Estimation of apparent digestibility coefficients 115

Analyses 115

Intestine and hepatopancreas morphology 116

Challenge test 117

Statistical analysis 117

3. Results 117

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

4. Discussion 125
References 129

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JEJU NATIONAL UNIVERSITY LIBRARY

List of tables

Table 2. 1. Formulation and proximate composition of the experimental diets for red sea bream (%, dry matter basis)....28

Table 2. 2. Amino acid composition (% in sample) of the experimental diets for red seabream 29

Table 2. 3. Growth performance and feed utilization of red sea bream fed the five experimental diets for 12 weeks. 41

Table 2. 4. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for red sea bream. 42

Table 2. 5. Apparent digestibility coefficients (%, ADC) for amino acid of the experimental diets for red sea bream. 42

Table 2. 6. Hematological parameters of red sea bream fed the five experimental diets for 12 weeks. 43

Table 2. 7. Non-specific immune response of red sea bream fed the five experimental diets for 12 weeks. 44

Table 2. 8. Proximate composition of red sea bream without viscera fed the five experimental diets for 12 weeks. 45

Table 2. 9. Visceral composition of red sea bream fed the five experimental diets for 12 weeks. 45

Table 2. 10. Morphometric and biometric parameters of red sea bream intestine fed the five experimental diets for 12 weeks. 46

Table 2. 11. Liver lipid and muscle immunological parameters of red sea bream fed the five experimental diets for 12 weeks. 47

Table 3. 1. Formulation and proximate composition of the experimental diets for red sea bream (%, dry matter basis) 55

Table 3. 2. Amino acid composition (% in sample) of the experimental diets for red seabream 56

Table 3. 3. The molecular weight distribution, proximate and essential amino acids composition of protein hydrolysates (from product technical data sheets)....57

Table 3. 4. Growth performance and feed utilization of red sea bream fed the five experimental diets for 12 weeks....64

Table 3. 5. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for red sea bream. 64

Table 3. 6. Apparent digestibility coefficients (%, ADC) for amino acid of the experimental

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

diets for red sea bream. 65

Table 3. 7. Hematological parameters of red sea bream fed the five experimental diets for 17 weeks. 65

Table 3. 8. Plasma HDL, LDL levels, muscle MDA level and TAC of red seabream fed the five experimental diets for 17 weeks. 66

Table 3. 9. Non-specific immune response of red sea bream fed the five experimental diets for 17 weeks. 66

Table 3. 10. Proximate composition of red sea bream fed the five experimental diets for 17 weeks. 67

Table 3. 11. Viscera proximate composition, liver lipid content and muscle water retention (%, wet basis) of red seabream fed the five experimental diets for 17 weeks. 67

Table 3. 12. Whole-body eviscerated carcass proximate composition (%, dry matter) of red seabream fed the fivet experimental diets for 17 weeks. 67

Table 3. 13. Morphometric parameters of red sea bream intestine fed the five experimental diets for 12 weeks..68

Table 3. 14. Biometric parameters of red seabream intestine fed the fivet experimental diets for 17 weeks. 68

Table 4. 1 Formulation and proximate composition of the four experimental diets for red seabream (%, dry matter basis)..76

Table 4. 2. The molecular weight distribution of shrimp hydrolysate (SH) (%). 77

Table 4. 3. Proximate composition of shrimp hydrolysate (SH) and krill meal (KM) (from product technical data sheets). 77

Table 4. 4. Growth performance and feed utilization of red seabream fed the four experimental diets for 15 weeks 82

Table 4. 5. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for red seabream. 83

Table 4. 6. Hematological parameters of red seabream fed the four experimental diets for 15 weeks. 83

Table 4. 7. Non-specific immune response of red seabream fed the four experimental diets for 15 weeks. 84

Table 4. 8. Whole-body composition of red seabream fed the four experimental diets for 15 weeks (%, DM). 84

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Table 5. 1. Formulation of the four experimental diets for Pacific white shrimp (%, dry matter basis). 94

Table 5. 2. Amino acid composition (% in sample) of the four experimental diets for Pacific white shrimp. 95

Table 5. 3. Growth performance and feed utilization of Pacific white shrimp fed the six experimental diets for 52 days. 102

Table 5. 4. Non-specific immune parameters of Pacific white shrimp fed the six experimental diets for 52 days. 102

Table 5. 5. Hematological parameters of Pacific white shrimp fed the six experimental diets for 52 days. 103

Table 5. 6. Whole body composition (%) without viscera of experimental Pacific white shrimp fed the six experimental diets for 52 days. 103

Table 5. 7. Amino acid composition (% in sample) for whole body of experimental Pacific white shrimp fed the experimental diets for 52 days. 104

Table 5. 8. Intestinal morphology of Pacific white shrimp fed six experimental diets for 52 days. 104

Table 5. 9. Apparent digestibility coefficients (%, ADC) for dry matter, protein and lipid of the experimental diets for Pacific white shrimp. 105

Table 5. 10. Apparent digestibility coefficients (%, of ADC) for amino acid composition of the six experimental diets for red seabream. 105

Table 6. 1. Formulation of the seven experimental diets for Pacific white shrimp (%, dry matter basis). 112

Table 6. 2. Amino acid composition (% in sample) of the seven experimental diets for Pacific white shrimp. 113

Table 6. 3. Growth performance and feed utilization of Pacific white shrimp fed the seven experimental diets for 52 days. 120

Table 6. 4. Non-specific immune parameters of Pacific white shrimp fed the seven experimental diets for 52 days. 121

Table 6. 5. Hematological parameters of Pacific white shrimp fed the seven experimental diets for 52 days. 122

Table 6. 6. Whole body composition (%) Pacific white shrimp fed the seven experimental diets for 10 weeks. 123

Table 6. 7. Intestinal morphology of Pacific white shrimp fed seven experimental diets for 52 days. 123

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Table 6. 8. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for Pacific white shrimp. 124

Table 6. 9. Apparent digestibility coefficients (%, ADC) for amino acid of the experimental diets for Pacific white shrimp. 124

List of figures

Figure 2. 1. Liver insulin-like growth factors I mRNA expression for each diet group expressed as a ratio to HFM diet values. 48

Figure 2. 2. Survival rate of red sea bream fed five experimental diets for 12 weeks after challenge with *E. tarda.* 48

Figure 3. 1. Survival rate of red sea bream fed five experimental diets for 17weeks after challenge with *E. tarda.* 69

Figure 4. 1. Liver insulin-like growth factors I mRNA expression for each diet group expressed as a ratio to HFM diet values.. 85

Figure 4. 1. Liver insulin-like growth factors I mRNA expression for each diet group expressed as a ratio to HFM diet values.. 85

Figure 5. 1. Survival rate of Pacific white shrimp fed the six experimental diets after challenge with *Vibrio harveyi*. 106

Figure 6. 1. Survival rate of Pacific white shrimp fed the seven experimental diets after challenge with *Vibrio harveyi*. 125

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Chapter I

Literature review

1. Protein sources used in aquafeed

Protein is, a class of nitrogenous organic compounds that consist of large molecules composed of one or more long chains of amino acids (AA), an important and a major component of every cell in animals including fish. Protein is used to build and repair tissues. It is used to produce enzymes, hormones, and other body chemicals. Also, Protein is a building block of bones, cartilage, muscles, skin and blood. Fish and shrimp require 30-50% protein in their diet for optimal growth (Wilson et al., 1986) and they are unable to synthesize all AAs for the requirement. Therefore, fish and shrimp feeds are supplemented with protein sources that consist of high levels of protein and essential amino acids. Also, they are known as fastidious eaters those require a higher level of dietary proteins. Moreover, carnivorous and omnivores species require animal origin proteins for better growth performance. In this regard, fish meal (FM) is used as an ideal protein source for fish and crustaceans diets due to the high protein level and suitable AA proportion. Additionally, animal byproduct meals such as meat meal, poultry by-product meal, blood meal, krill meal and squid meal, as well as plant protein sources are used to supply protein in fish and shrimp diets.

Fish meal

FM is produced by fish or fishery byproducts following key processing steps such as cooking, pressing, drying and grinding. FM contains high levels of digestible proteins, essential AAs, essential fatty acids, minerals and vitamins those are meet the requirement of fish and crustaceans (Tacon et al., 2009). The protein level of high-quality FM is in the range between 60-72% by weight. However, the protein and AA content of FM varies with the

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species, freshness of raw materials and seasonal differences of the production (Romero et al., 1994; Aksnes and Mundheim, 1997; Nates, 2015). Therefore, FM divided into several grades such as prime/high-quality FM and standard quality FM. The quality of FM is a main factor that decides the use of other ingredients in fish diets. For instant, the lipid level of diet for gilthead seabream can be reduced when diets were supplemented with a high-quality FM (Vergara et al., 1999). However, world FM production was fairly stable for the last few decades. Therefore, High demand and price of FM are leading to use cost-efficient alternative protein source for fish and shrimp feed to formulate low FM diets which have become an inevitable research area in the fish feed industry.

Krill meal

Krill are small crustaceans classified into order Euphausiacea. They are one of the primary food sources for large marine mammals and fishes (Hardy, 2008). Also, they are a rich source of marine proteins, omega 3 fatty acids, phospholipids and astaxanthin. Therefore, krill processing by-products and by-catch are used to produce valuable products such as krill meal (KM), krill hydrolysates and krill oil (Tilseth and Hostmark, 2009). It is reported that KM can replace FM protein in a high plant protein diet for Pacific white shrimp (PWS) without sacrificing growth and feed utilization (Nunes et al., 2011). It is a good palatability enhancer that contains astaxanthin, chitin and chitosan in addition to suitable lipid and mineral profile for fishes (Goto et al., 2001; Hansen et al., 2010). Therefore, increased feed intake reported in some fish species when diets are incorporated with KM (Yoshitomi et al., 2006; Hatlen et al., 2017). Furthermore, it has been proved that dietary KM supplementation results in increased growth performance, feed utilization, health status digestibility and disease resistance of fish (Gaber 2005; Hansen et al., 2010; Yan et al., 2018). Generally, Krill

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meal includes as a feed ingredient when formulating low FM diets for red seabream (Takagi et al., 2001; Kader et al., 2012) Earlier, Shimizu and Chiaki, 1990 reported that feeding behavior of red seabreams can be stimulated by dietary KM supplementation. Later, Kader et al, (2010) replaced a high proportion of FM with soy protein concentrate (SPC) and KM in red seabream diet without compromising feeding behavior, growth and health. Recently Cho et al. (2018) tested krill meal as the main protein source in a red seabream diet and observed that amino acid profile and fatty acid profile of fish were improved by krill meal. Hydrolyzed krill products have been supplemented in red seabream diet to examine fish performances (Bui et al., 2014, Khosravi et al., 2015, 2017). Suresh et al. (2011) reported that diet attractability, palatability and growth of blue shrimp (*Litopenaeus stylirostris*) were improved by 3% KM supplementation. Therefore, KM is an effective protein source for fish and shrimp feed.

Squid liver powder

Squid meal, liver powder and viscera meal, byproduct of squid or cuttlefish processes, is appetizer and feed attractant used in fish and shrimp diet (Liang et al., 2000; Suresh et al., 2011). Growth and feed utilization of fish and shrimp can be improved by dietary supplementation of ingredients produced by squid processing by-products. Growth of Japanese seabass (*Lateolabrax japonicas*) was improved by dietary squid viscera meal supplementation compared to a high FM diet (Mai et al., 2006). Growth, trypsin activity and chymotrypsin activity of PWS were increased by dietary squid meal supplementation up to 9% and decreased when 15% of total protein was supplemented with squid meal in the diet (Córdova-Murueta et al., 2002). Dietary squid meal enhanced the growth of giant tiger prawn (*Penaeus monodon*) (Smith et al., 2005). Cruz-Ricque et al. 1989 revealed a positive effect of

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squid extract on growth, feed utilization and nutrient absorbance of Kuruma prawn (*Penaeus japonicas*). However, high levels of dietary squid product supplementation were reported to suppress shrimp growth (Córdova-Murueta et al., 2002; Smith et al., 2005). Also, it was suggested that long term feeding of squid product may result in cadmium accumulation in animal tissues (Mai et al., 2006).

Dried Fish soluble

Fish soluble is a by-product generated during fish manufacturing processes such as canning, FM and fish oil production. The soluble fraction is centrifuged to remove oil fraction and then use the water containing fraction to produce condensed or dried fish soluble (DFS) after drying. As a feed ingredient in aquaculture, It was reported that fish soluble supplementation to red drum (*Sciuenops ocellatus*) diets was beneficial to improve growth and feed utilization (McGoogan and Gatlin, 1997). Also, fish soluble is recommended to use in high plant protein diets for Kuruma shrimp to replace FM and improve growth performance.

2. Plant protein sources as an alternative to fish meal

Protein units of plant protein sources (PPS) are low in cost and consistently available in the market compared to FM protein. Therefore, in the early 1980s, plant protein ingredients, such as soybean meal and corn gluten meal, were used in the animal feed industry as less expensive alternatives. With the increase of demand and price of FM, researchers have focused on plant origin protein sources as an alternative to FM. However, plant origin feed ingredients contain lower protein levels compared to those of animal origin protein sources. Plant-derived proteins are deficient in several essential amino acids including lysine, methionine and tryptophan. In some cases, PPSs contain offensive compounds to the

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olfactory receptors of fish and shrimp. Therefore, palatability of diets could be reduced due to PPS causing lower feed intake. Moreover, anti-nutritional and toxic factors, such as glycoside, phytates, mycotoxin, trypsin inhibitors, gossypol, tannin, are present in PPS to reduce growth and immunity of fish and shrimp. In a study, gilthead seabream was fed with a FM diet and a plant protein diet. The growth performance of fish was not improved compared to a high FM diet although the plant protein diet was formulated to contain the same AA levels to FM diet. Also, feed intake was decreased in fish fed plant protein diet (Gómez-Requeni et al., 2004). Lower palatability, anti-nutritional factors and high level of carbohydrates in PPS are the possible reason for these results. Further, it was reported that dietary AA, such as lysine (Cheng et al., 2003; Xie et al., 2012), methionine (Webster et al., 1995; Gu et al., 2013) and taurine (Li et al., 2016), supplementation with PPS to fish and shrimp diets improve growth and feed utilization. Therefore, recent studies on PPS supplemented diet proved that FM replacement with PPS and AAs is a suitable way to alleviate the adverse effects of PPS and reduce FM level in diets without sacrificing fish and shrimp performances.

3. Development of low fishmeal diets

Fish and shrimp, especially carnivorous and omnivorous species, are unable to adapt diets prepared by using PPS as the sole protein source without compromising growth and immunity. Therefore, to prevent this problem, great effort has been made to develop low FM diets for fish and shrimp over the past decades. FM was replaced with animal protein sources, such as poultry byproduct meal, blood meal, meat and bone meal and feather meal in several studies (Steffens, 1994; Millamena, 2002; Wang et al., 2006; Yang et al., 2004; Cruz-Suárez et al., 2007). However, animal proteins are costly compared to PPS and, PPSs are abundant and low in cost. Therefore, in the case of fish and shrimp, researchers afforded to develop

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LFM diets with PPS. For red seabreams (*Pagrus major*), A mixture of fermented soybean meal and scallop by-product blend replaced 30% of FM in a diet for juvenile fish without scarifying growth, body composition and health (Kader et al., 2011). SPC with krill meal, SM and fish soluble replaced 50% of FM from the diet maintaining better growth and health status of juvenile RSB compared to a 60% FM diet (Kader et al., 2011). Also, several attempts were made to produce LFM diets for shrimps (Amaya et al., 2007: Hernández et al., 2008; Liu et al., 2011; Xie et al., 2019).

4. Protein hydrolysates

Protein hydrolysates, produced by enzymolysis of protein sources, have been used as feed ingredients in fish diets due to their excellent functional properties (palatability and health enhancement) and nutritive values (high digestibility) (Khosravi et al., 2015; Xu et al., 2016). Many studies have confirmed that during the process of hydrolysis, source proteins are broken down into palatable AAs and uneven sized peptides that are associated with antimicrobial, antihypertensive, antioxidant and immunomodulatory activities (Bougatef et al., 2010; Chalamaiah et al., 2012; Song et al., 2012; Sila et al., 2014; Ennaas et al., 2015;). Moreover, protein hydrolysates are reported to upregulate gene expression of cholecystokinin, peptide transporter 1, trypsin and insulin-like growth factor 1 in fish (Zheng et al., 2012; Cai et al., 2015; Chalamaiah et al., 2018). Thereby, it has been suggested that protein hydrolysates are associated with improved digestion, nutrient absorption and somatic growth.

5. Marine Protein hydrolysates

Marine protein hydrolysate (MPH) is a breakdown product of fish proteins containing smaller peptides and amino acids. MPH is obtained by treatment of fish meat with trypsin, alcalase, chymotrypsin, pepsin, or other enzymes under controlled conditions of pH and

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temperatures. MPH normally has a high content of protein primarily due to the solubilization of the protein during the hydrolysis reaction and removal of non-protein compounds such as fat and other insoluble materials. In some cases, the MPH has also been subjected to a drying step (Dong et al., 2008; Chalamaiah et al., 2012). MPHs contain all of the essential amino acids, making them a good nutritional product. The amino acid composition of the MPH product can affect its bioactive and functional properties. The amino acid composition of the MPH is affected by the enzymatic reaction parameters such as the type of enzyme, pH value, and time (Bhaskar et al., 2008). The enzymatic hydrolysis influences the protein functional properties by changing the peptides and amino acid composition of the proteins. Peptides isolated from various fish protein hydrolysates have shown different biological activities such as antihypertensive, antioxidative, antithrombotic, and immunomodulatory activities. Extensive studies have shown that fish protein hydrolysates have good nutritional properties and thus can be used widely in the food industry for various purposes. These include texturing, gelling, foaming, emulsification, protein supplements, flavor enhancers, surimi production, and beverage stabilizers (Kristinsson, 2007). Therefore, MPH can be used as a suitable feed ingredient in fish and shrimp diet.

Shrimp hydrolysates

Shrimp hydrolysates are produced from shrimp byproduct or processing waste such as shrimp heads, cuticles or tail muscle. Especially, shrimp cephalothorax and exoskeleton which known as a rich source of chitin, astaxanthin and protein, are account for 35-45% of whole body weight as inedible waste (Meyers 1986; Shahidi & Synowiecki, 1991). Therefore, several methods were used to exploit that byproduct to edible and valuable products and hydrolysis was recommended as a successful way to convert by-product into micronutrient

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(Nwanna, 2003; Lopez-Cervantes et al., 2006; Bueno-Solano et al., 2009; Cahu et al., 2012). The studies on SH revealed those antioxidative, anti-hypertensive, antimicrobial and myotropic activities as biological properties of SH (Nii et al., 2008; Huang et al 2011; Kleekayai et al., 2015; Leduc at el., 2018). Further, a trace amount of heavy metals such as Hg, Pb and Cd were recorded in shrimp by products while free amino acid content was 15% higher than the edible part of a shrimp (Heu et al., 2003). In this regards, SH has been evaluated in diet for several fish and shrimp species and positive effects were elucidated on growth, immunity, digestibility, palatability and health of fish (Plascencia-Jatomea at el., 2002; Leal at el., 2010; Khosravi et al., 2015a; Khosravi et al., 2015b; Khosravi et al., 2017; Leduc at el., 2018).

Tilapia hydrolysates

Tilapia hydrolysate (TH) is a good source of essential AAs and minerals (Foh et al., 2011; Silva et al., 2014). Thousands of different peptides have been identified in TH those have antimicrobial properties (Robert et al., 2015). Also, there is evidence of the improved growth performance, protein digestibility, health and disease resistance of red seabream fed 5% of FM replaced diet with TH (Bui et al., 2014).

Salmon hydrolysates

Salmon processing byproducts such as head, intestine, frame and pectoral fin were largely generated during salmon processing. Among them, pectoral fin byproduct contains a lot of proteins, which are a good source for bioactive peptides. Ahn et al. (2012) observed that salmon protein hydrolysates showed no cytotoxic effects on macrophage cells, and it significantly inhibited intracellular reactive oxygen species generation, lipid peroxidation, and enhanced the level of glutathione. Additionally, Salmon protein hydrolysates showed

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anti-inflammatory activity by inhibiting nitric oxide production in macrophage cells.

Tuna hydrolysates

Tuna hydrolysates (TuH) are produced from fish processing waste (viscera, head or frame) which are rich in protein and well-balanced amino acid. In the case of fish, the amount of waste is depending on the processing method. Production of canned tuna generates 50 to 70% of raw material as solid waste (Saidi et al., 2014). Therefore, different types of processing waste are available and, used to produce tuna hydrolysates (Guerads et al., 2002; Nguyen et al., 2012; Cheng et al., 2015). Hydrolysates from tuna liver showed antioxidant and antihypertensive properties indicating that it is beneficial as a food ingredient (Je et al., 2009). Tuna frame protein hydrolysate is a beneficial ingredient which contains peptides to act as nutraceuticals and pharmaceuticals against diseases related to hypertension (Lee et al., 2010). Similarly, hydrolysates from bigeye tuna (*Thunnus obesus*) dark muscle also beneficial against hypertension (Qian et al., 2007). Herpandy et al. (2011) reported that tuna hydrolysate exhibits functional properties (whipping, gelling and texturing) as a food ingredient. Therefore, TuH is used as a protein source in fish and shrimp diet due to those properties as well as valuable protein and AA content. In a previous study, we observed that TuH improve growth, feed utilization, immunity, digestibility and disease resistance of red seabreams (Pagrus major) (Khosravi et al., 2015). In shrimp, growth was improved by dietary tuna head hydrolysate supplementation (Nguyen et al., 2012). Hernández et al. (2011) identified that tuna by-product hydrolysates provide an attractability to shrimp feed while improving protein digestibility and amino acid profile of feed. Also, they observed that the growth performance of fish positively influenced after six weeks of feeding trial.

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Krill hydrolysates

Krill hydrolysates (KH), produced by enzymatically hydrolyzed krill, are tested as a feed ingredient during past few decades. A technology to recover krill protein by autoproteolysis was developed by Kolakowski et al. (1980) as a record for use of KH and, later, endogenous enzymes of krill were used for the proteolysis process (Kolakowski and Gajowiecki 1992). KH is a feed attractant growth promoter for fish (Kolkovski et al., 2000). Kousoulaki et al. (2013) observed that dietary KH supplementation stimulated the appetite of Atlantic salmon (*Salmo salar*). Peptide powder prepared by hydrolyzing tail muscle of Antarctic krill was identified as a therapeutic agent for hypertension (Hatanaka et al., 2009). KH included soybean based diet resulted in high body weight, survival and shorter molting cycle of juvenile American lobster, (*Homarus americanus*) (Floreto et al., 2008). Also, in a previous study, we observed that growth performance, feed utilization, intestine morphology, immunity and disease resistance of red seabreams and olive flounder were improved by dietary KH supplementation (Khosravi et al., 2015, 2017).

6. Chapter justification

The studies presented in this dissertation are aimed to evaluate supplementary effects of MPHs in low fish meal diets on growth performance, feed utilization, innate immunity, hematological parameters, diet digestibility gut morphology and disease resistance of red seabream or PWS.

Chapter two was conducted to evaluate the effects of salmon protein hydrolysates, tilapia protein hydrolysates and tuna protein hydrolysate supplemented low fish meal diet in red seabream diets. Two control diets, high fish meal (HFM) and low fish meal (LFM), were formulated as positive and negative controls to compare the effects of hydrolysate products.

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The HFM diet contained 36.8% FM. The LFM diet was supplemented with SPC by replacing 63% of FM from the HFM diet to contain 13.5% FM. Tuna protein hydrolysate was coated to the LFM diet.

In chapter three, supplemental effects of tilapia hydrolysates and/or shrimp hydrolysates in the red seabream diet were evaluated by this chapter. FM level of HFM diet was increased to 37% and LFM diet was formulated by replacing 50% of FM from HFM diets with SPC to contain 18.5% FM.

In chapter four, supplementary effects of shrimp protein hydrolysate or krill meal in a LFM diet was compared for red seabream. In this chapter, HFM diet was formulated including 40% FM and LFM diet was prepared to contain 25% FM by replacing 37.5% of fish meal with SPC from the HFM diet. Shrimp protein hydrolysates or krill meal diets were formulated by substituting 12.5% of fish from LFM diet to contain 50% of FM compared to HFM diet.

In chapter five, the effects of tuna protein hydrolysates, shrimp protein hydrolysates, krill protein hydrolysates and dried fish soluble were examined on growth, feed utilization, intestinal morphology, innate immunity and disease resistance of PWS. Squid liver powder or krill meal supplemented diets were used as control diets. All the diets were formulated to contain 9.1% tuna meal as a marine protein source and high proportion of soybean meal was incorporated as the main protein source.

In chapter six, the effects of different dietary levels of dried fish soluble, shrimp protein hydrolysates and tuna protein hydrolysates were evaluated in diets for PWS. Similar to chapter six, a diet supplemented with squid liver powder was used as the control diet. All the

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diets were formulated to contain 9.1% tuna meal as a marine protein source and high proportion of soybean meal was incorporated as the main protein source.

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Chapter II

Dietary supplementation of different protein hydrolysates products in low fish meal diets on growth, innate immunity and disease resistance of red seabream (*Pagrus major*).

1. Introduction

It is an essential practice in aquafeed industry to develop alternative protein sources for FM. The use of plant protein sources in exchange of FM is a novel trend to prevent the excessive use of FM (Hardy, 2008).

MPHs are products of hydrolysis reaction of proteins and result in shorter peptides or amino acids. They are easily absorbed by animals due to low molecular weight compounds and well-balanced amino acid profiles (Wisuthiphaet et al., 2015). Hydrolysates have been used as FM replacer in fish and shrimp diets over the last few decades and positive effects of MPH on fish species have been reported as an ingredient to replace FM improving immunity and disease resistance. (Kolkovski et al., 2000; Zambonino Infante, 2001; Cahu and; Refstie et al., 2004; Aksnes et al., 2006a; Aksnes et al., 2006b; Khosravi et al., 2017) ,

Salmon processing byproducts such as head, intestine, frame and fins are largely generated during salmon processing. Among them, pectoral fin byproduct contains high level of proteins, which are good source for bioactive peptides. Ahn et al. (2012) observed that salmon protein hydrolysates showed no cytotoxic effects on macrophage cells, and it significantly inhibited intracellular reactive oxygen species generation and, lipid peroxidation, and enhanced the level of glutathione. Tilapia hydrolysate (TH) is a good source of essential

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AAs and minerals (Foh et al., 2011; Silva et al., 2014). Thousands different peptides have been identified in TH that have antimicrobial properties (Robert et al., 2015). Also, there is evidence of the improved growth performance, protein digestibility, health and disease resistance of red seabream fed a 5% of FM replaced diet with TH (Bui et al., 2014). Tuna hydrolysates (TuH) are produced from fish processing wastes which are rich sources of protein and well balanced amino acids (Guerads et al., 2002; Nguyen et al., 2012; Cheng et al., 2015). Khosravi et al. (2015) observed that TuH improved growth, feed utilization, immunity, digestibility and disease resistance of red seabreams.

In our previous studies, positive effects on fish growth, feed utilization, innate immunity, intestine histology, digestibility, disease resistance against pathogens for olive flounder and red seabream were observed by partial replacement of FM with MPHs (Khosravi et al., 2015; Bui et al., 2014; Khosravi et al., 2017). Therefore, this study was conducted to examine the effects of salmon protein hydrolysates, tilapia protein hydrolysates and tuna protein hydrolysates on growth performance, feed utilization, intestinal histology, digestibility, innate immunity, hematological parameters, liver IGF-1 expression and disease resistance against *Edwerdsiella tarda* of red seabream.

2. Materials and Methods

Experimental diets

Five experimental diets were formulated to be isonitrogenous (36.8% crude protein) and isocaloric (18 kJ g^{-1}) (Table 1.1) by inclusion of salmon protein hydrolysates, tilapia protein hydrolysates and tuna protein hydrolysates provided by AQUATIV (Aquaculture Division of DIANA, Member of SYMRISE Group), Elven, France. A FM-based diet (36.8%) was regarded as a high FM

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diet (HFM) and a diet contained SPC, as a substitute for FM, at replacement level of 63.3% was considered as low-FM diet (LFM). Three other experimental diets were prepared by dietary inclusion of salmon protein hydrolysate powder and tilapia protein hydrolysate powder to low FM based diet at 5% inclusion level and one other diet was prepared by coating 2% (in wet basis) tuna hydrolysate liquid to LFM diet using a hand sprayer (SalH, TH and TuH respectively).

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Table 2. 1. Formulation and proximate composition of the experimental diets for red seabream (%, dry matter basis)

Ingredients	Experimental diets				
	HFM	LFM	SalH	TH	TuH
Fish meal	36.8	13.5	13.5	13.5	13.5
Salmon hydrolysate			4.51		
Tilapia hydrolysate				4.81	
Tuna hydrolysate					2.00
Soy protein concentrate (SPC)	10.1	28.5	24.0	24.0	28.5
Corn gluten meal	12.0	12.0	12.0	12.0	12.0
Wheat flour	28.7	29.0	29.3	29.0	29.0
Squid liver oil	4.90	6.20	6.20	5.70	6.20
Soybean oil	4.00	4.00	4.00	4.00	4.00
Mineral Mix[1]	1.00	1.00	1.00	1.00	1.00
Vitamin Mix[2]	1.00	1.00	1.00	1.00	1.00
Starch	1.00	1.60	1.30	1.80	1.60
Choline chloride	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.00	0.50	0.50	0.50	0.50
L-Methionine	0.00	0.20	0.20	0.20	0.20
Taurine	0.00	0.50	0.50	0.50	0.50
Di-calcium phosphate	0.00	1.50	1.50	1.50	1.50
*Proximate composition					
Crude protein	44.9	44.9	44.9	44.9	44.9
Crude lipid	14.0	14.0	14.0	14.0	14.0
Crude ash	9.58	5.41	5.45	5.35	5.41
Moisture	6.42	6.61	6.57	6.53	6.69

[1]Mineral premix (g kg^{-1} of mixture): $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3$. $6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[2] Vitamin premix (g kg^{-1} of mixture): L-ascorbic acid, 121.2; DL-α tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-$_D$-pantothenate, 12.7; myo-inositol, 181.8; $_D$-biotin, 0.27; folic acid, 0.68; p-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

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Table 2. 2. Amino acid composition (% in sample) of the experimental diets for red seabream

Amino acid	Experimental diets				
	HFM	LFM	SalH	TH	TuH
Aspartic Acid	4.37	4.61	4.59	4.94	5.01
Threonine	1.85	1.79	1.73	1.85	1.80
Serin	2.05	2.26	2.12	2.30	2.21
Glutamic Acid	8.55	9.62	9.00	10.3	10.1
Proline	3.08	3.12	3.00	2.48	2.73
Glycine	2.33	2.03	2.22	2.45	2.28
Alanine	2.95	2.62	2.54	2.22	2.19
Valine	2.43	2.44	2.29	2.39	2.42
Isoleucine	2.12	2.33	2.08	2.22	2.27
Leucine	4.36	4.56	4.08	3.69	3.66
Tyrosine	1.42	1.55	1.39	1.48	1.36
Phenylalanine	2.28	2.50	2.28	2.49	2.46
Histidine	1.68	1.52	1.53	1.70	1.78
Lysine	2.92	2.99	2.94	2.77	2.80
Arginine	2.78	2.93	2.89	3.30	3.41

Fish and feeding trial

Juvenile red seabream were transported from a private hatchery to the Marine and environmental research institute, Jeju National University, Jeju, Korea. All fish were acclimatized to experimental conditions and facilities for two weeks while feeding a commercial diet. Six hundred fish (Initial mean body weight, 13.8±0.04g) were randomly distributed into 24 polyvinyl circular tanks (40 fish / tank). Each tank was randomly assigned to one of the three replicates of five dietary treatments and supplied with filtered sea water at a flow rate of 3 L min^{-1} and aerated with sandstones to maintain sufficient dissolved oxygen. Fish were fed the experimental diets twice a day (09.00 and 18.00 h) until satiation for 12

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weeks. Growth checking was conducted at three weeks interval. Feeding was stopped 24 hour prior to weighting to minimize stress. Uneaten feed was collected 30 min after feeding and reweighed to determine feed intake.

At the end of the feeding trial, all the fish from each tank collectively weighted to obtain total biomass. Four fish from each tank were randomly selected, anesthetized with 2-Phenoxy methanol solution (200 ppm) and blood was collected from caudal vein with heparinized syringes for determine hematocrit and hemoglobin. Then plasma were separated by centrifugation at 5000×g for 10 min and stored at -70 ◦C until analyze total immunoglobulin (Ig), anti-protease activity and biochemical parameters. Another set of blood samples were taken from the caudal vein of four fish from each tank using non heparinized syringes, allowed to clot at room temperature for 30 min and the serum was separated by centrifugation for 10 min at 5000×g and stored at -70 ◦C for analysis of innate immune response parameters.

Estimation of apparent digestibility coefficients

For estimation of apparent digestibility coefficient of the experimental diets, chromic oxide (Cr_2O_3) (Sigma-Aldrich, St. Louis, USA) was included in the diets as an inert indicator at a concentration of 1.0%. All dry ingredients were thoroughly mixed and extruded through a pelletizer machine (SP-50, Gum Gang Engineering, Daegu, Korea) to ideal size after addition of fish oil, soybean oil and 10% double distilled water. The pellets were dried with electric fans at room temperature and stored at -20 ℃ until used.

Fecal collection

New sets of red seabream (initial body weight, 80g) were distributed into 300 L

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capacity Guelph system (fecal collection system) tanks at a density of 100 fish per tank. The tanks were supplied with cartridge-filtered seawater at a flow rate of 1 L min^{-1} and aeration to maintain enough dissolved oxygen. Fish were hand-fed one of the test diets to apparent satiation once daily at 18:00h. One hour after feeding, the rearing tanks were brushed out to remove uneaten feed and fecal residues. On the next day, feces were collected from the fecal collection columns at 09:00h. A secondary fecal collection tube, under the collection tank, was additionally installed to collect all the possible feces. The collected feces in the tube was separated from supernatant water using a disposable paper filter and stored at -40 ℃. Then the fecal samples were freeze-dried for 24 h and stored at -20 ℃ until analyzed.

Chromium oxide analyses

Chromium oxide content of diet and feces samples were analyzed by the method described by Divakaran et al. (2002). Briefly, a known weight (5-10 mg) of ash samples of either diet or feces containing chromium oxide was placed in glass test tubes. Then 4 ml of perchloric reagent was added along the sides of the test tube to wash down any adhering ash. Perchloric reagent was prepared as follows: two hundred milliliter of concentrated nitric acid was added to 100 ml of distilled water, cooled down and then 200 ml perchloric acid (70%) was added. The test tubes were set in a heating block and heated at 300 ℃ for 20 min, for oxidation of chromium oxide to monochromate (CrO_4^{2-}). Then the tubes were cooled down to room temperature and their contents were quantitatively transferred and made up to 25 ml in a volumetric flask by rinsing repeatedly with distilled water. The absorbance of samples was read at 350 nm using a spectrophotometer (Beckman DU-730, USA). A known weight (2 – 4 mg) of chromium oxide was similarly treated and used as standard.

Apparent digestibility estimations

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The Apparent digestibility coefficients for dry matter and protein of the experimental diets were calculated by the following formula:

ADC of dry matter (%) = 100 - 100 × (% Cr_2O_3 in diet / % Cr_2O_3 in feces)

ADC of protein (%) = 100 - 100 × (% Cr_2O_3 in diet / % Cr_2O_3 in feces) × (%protein in feces /%protein in diet)

Analyses

Proximate composition analysis

Analysis of moisture and ash content were performed by the standard procedures (AOAC, 1995). Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FOSS, Sweden) and crude lipid was determined using Soxhlet Extraction System C-SH6 (Korea).

Hematological parameters

Hematocrit was determined by microhematocrit technique (Brown, 1980). Hemoglobin and plasma levels of glucose, total protein and cholesterol were determined by an automated blood analyzer (SLIM, SEAC Inc, Florence, Italy).

Immunological assays

The oxidative radical production by phagocytes during respiratory burst was measured through NBT assay described by Anderson and Siwicki (1995). Briefly, blood and nitro-blue-tetrazolium (0.2%) (NBT; Sigma, USA) were mixed in equal proportion (1:1), incubated for 30 min at room temperature, then 50 µl was taken out and dispensed into glass tubes. Then, 1 ml of dimethylformamide (Sigma, USA) was added and centrifuged at 2000 g

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for 5 min. Finally, the optical density of supernatant was measured at 540 nm using spectrophotometer. Dimethylformamide was used as blank.

Plasma immunoglobulin (Ig) levels were determined according to the method described by Siwicki and Anderson (1993). Briefly, plasma total protein content was measured using a micro protein determination method (C-690; Sigma), prior to and after precipitating down the immunoglobulin molecules, using a 12% solution of polyethylene glycol (Sigma). The difference in protein content represents the Ig content.

A turbidometric assay was used for determination of serum lysozyme level through the method described by Hultmark (1980) with slight modifications. Briefly, *Micrococcus lysodeikticus* (0.75 mg ml^{-1}) was suspended in sodium phosphate buffer (0.1 M, pH 6.4) then 200 μl of suspension was placed in each well of 96-well plates and 20 μl of serum was added subsequently. The reduction in absorbance of samples was recorded at 570 nm after incubation at room temperature for 0 and 30 min in a microplate reader. Hen egg white lysozyme (Sigma) was used as a standard. The values were expressed as μg ml^{-1}.

Serum myeloperoxidase (MPO) activity was measured according to Quade and Roth (1997). Briefly, twenty microliter of serum was diluted with HBSS (Hanks Balanced Salt Solution) without Ca^{2+} or Mg^{2+} (Sigma, USA) in 96-well plates. Then, 35 μl of 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB, 20 mM) (Sigma, USA) and H_2O_2 (5 mM) were added. The color change reaction was stopped after 2 min by adding 35 μl of 4 M sulfuric acid. Finally, the optical density was read at 450 nm in a microplate reader.

Serum superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and

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xanthine oxidase using a SOD Assay Kit (Sigma, 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37 ℃. The percent inhibition was normalized by mg protein and presented as SOD activity units.

The plasma anti-protease activity was measured according to the method described by Ellis (1990), with slight modifications (Magnadóttir et al., 1999). Briefly, twenty microliter of serum was incubated with 20 µl of standard trypsin solution (Type II-S, from porcine pancreas, 5 mg ml^{-1}, Sigma-Aldrich) for 10 min at 22 °C. Then, 200 µl of phosphate buffer (0.1 M, pH 7.0) and 250 µlazocasein (2%) (Sigma-Aldrich) were added and incubated for 1 h at 22 ℃. Five hundred microliter of trichloro acetic acid (10%) (TCA) was added and further incubated for 30 min at 22 °C. The mixture was centrifuged at 6000×g for 5 min and 100 µl of the supernatant was transferred to the wells of a 96 well flat bottomed microplate containing 100 µl of NaOH (1 N). Optical density was read at 430 nm. For a 100% positive control, buffer was replaced for serum, while for the negative control buffer replaced both serum and trypsin. The trypsin inhibition percentage was calculated using the following equation:

Trypsin inhibition (%) = $(A_1 - A_2/A_1) \times 100$

Where A_1 = control trypsin activity (without serum); A_2 = activity of trypsin remained after serum addition.

GPx activity was assayed using kit (Biovision, Inc. California, USA). In this assay, cumene hydroperoxide was used as a peroxide substrate (ROOH), and glutathione reductase

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(GSSG-R) and NADPH (b-Nicotinamide Adenine Denucleotide Phosphate, Reduced) were included in the reaction mixture. The change in 340 nm due to NADPH oxidation was monitored and was indicative of GPx activity. Briefly, 50 µl of serum was added to 40 µl reaction mixture and incubated for 15 minutes and then 10 µl cumene hydroperoxide was added and OD1 read at 340 nm. After 5 min of incubation OD2 read in 340 nm by a microplate reader. Activity of GPx was calculated as mU ml^{-1}.

Catalase activity was assayed using kit (Biovision, Inc. California, USA). In this assay hydrogen peroxide used as the substrate and catalase react with H_2O_2 to produce water and oxygen. Briefly, 78 µl of serum as added to 50 µl of developer mix. After 30 minuts, oxired™ probe which was included in developer mix, react with unconverted H_2O_2 and produce a product which can be measured at 570nm by a microplate reader. Activity of catalase was calculated as mU ml^{-1}.

Muscle total antioxidant capacity.

Total antioxidant capacity (TAC) was determined using a commercial kit (Antioxidant assay kit, Sigma; CS0790). Samples (tissue lysate solutions) were prepared using an assay buffer solution provided with the kit. Then, the samples were added with mixture of ABTS (2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and hydrogen peroxide (150µl). Hydrogen peroxide oxidize ABTS and produce ABTS·+ (2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonate). After 10 minutes of incubation, samples were added with metmyoglobin solution (100µl). Metmyoglobin contain ferryl myoglobin which react with ABT+ and produce a green color soluble chromogen. Antioxidants in the samples suppress the production of the ABTS+ in a concentration dependent manner and the color intensity decreases proportionally. The color intessity was determined using a plate reader at 405 nm.

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TAC values of samples were expressed as an equivalent of the mmol concentration of a (±)-6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylix acid (Trolox) solution. Trolox standard curve at the range of 0.00 – 0.42 mM was prepared for the assay.

Muscle lipid peroxidation.

Muscle malondialdehyde (MDA) levels were determined spectrophotometrically using Lipid Peroxidation (MDA) Assay Kit (Biovision, Inc., Milpitas, CA, USA). Ten mg of muscle samples were homogenized on ice in MDA Lysis Buffer and Centrifuge at 13,000g for 10 minutes to remove insoluble material. Then, phosphoric acid, thiobarbituric acid (TBA) and sulfuric acid were added to 10 µl of tissue homogenate and incubated at 95 ℃ for 60 minutes. After cooling down to room temperature in a bath for 10 minutes, absorbance was measured at 532 nm. Standard curve was prepared using standard solution provided by kit and values were expressed as nmol/mg tissue.

Intestinal morphology

Another set of two fish per tank collected and the whole intestine removed and sampled for its histology. Body weight, liver weight, viscera weight intestine length and fish length were measured and recorded for calculation of CF, HSI and VSI. The intestine samples were fixed in Bouins solution, dehydrated in graded series of ethanol, embedded in paraffin and then sectioned in 5µm sagittal serial sections.

Slides were stained with Harris hematoxylin and 0.5% eosin for general histological observation and the Alcian blue (AB) at pH 2.5 and periodic acid Schiff (PAS) for observation of the mucus secreting goblet cells. The morphometric measurements of villus length (VL), intestine diameter (ID) and enterocyte height (EH) were made, using the ImageJ

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1.44 analysis software.

Expression levels of liver IGF-I mRNA

Liver total RNA was isolated using Trizol reagent (Sigma. T9424) and treated with RNase-free DNase (Omega Bio-Tek), following the manufacturer´s protocol. The quantity of the RNA was calculated using the absorbance at 260 nm. The integrity and relative quantity of RNA was checked by electrophoresis. PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa Code. DRR047) was used to remove genomic DNA and reverse transcription. One microgram of total RNA was reverse-transcribed into cDNA in a volume of 10 µL, containing 1 µL of gDNA Eraser and 2 µL of 5× gDNA Eraser Buffer. This mix was heated at 42 ℃ for 2 min. and then 4 µL of 5 ×PrimeScript Buffer (for Real Time), 1 µL of PrimeScript RT Enzyme Mix I, 1 µL of RT Primer Mix and 4 µL of RNase-free dH2O were added to a final volume of 20 µL. After incubation at 37 ℃ for 30 min, the reaction was stopped by heating to 85 ℃ for 5 s.

Levels of IGF-I transcript were measured by real-time PCR (SYBR Green I), using 18S rRNA as a housekeeping gene. Primers for real-time PCR were designed based on the previously cloned sequence for IGF-I (NCBI Genbank accession no: AY996779) and 18S rRNA (NCBI Genbank accession no: AB259837). Real-time RT-PCR was conducted by amplifying 2.0 µL of cDNA with TaKaRa SYBR Premix Ex Taq (Perfect Real Time) (TaKaRa Code.DRR041A) on a TaKaRa PCR Thermal Cycler Dice Real Time System (TaKaRa Code.TP800). Amplification conditions were as follows: 30 s at 95 ℃; 40 cycles of 5 s at 95 ℃, 30 s at 60 ℃. Melting curve analysis of amplification products was performed at the end of each PCR to confirm that a single PCR product was detected. Each sample was run in triplicate, and PCRs without the addition of the template were used as negative controls.

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Relative expression ratio of IGF-I was calculated based on the PCR efficiency (E) and the Ct of a sample versus the control (FM treatment) and expressed in comparison with the reference gene (18S rRNA), according to Pfaffl's mathematical model (Pfaffl, 2001).

$$Ratio = [(E_{IGF\text{-}I})^{\Delta Ct(control\text{-}sample)}]/[(E_{actin})Ct^{(control\text{-}sample)}]$$

Challenge test

At the end of the feeding trial, ten fish from each tank (30 fish per treatment) were intraperitoneally injected with *Edwardsiella tarda* (ATCC 15947, Korea Collection for Type Cultures) suspension (1×10^5 CFU ml^{-1}). *E. tarda* was provided by the Marine Applied Microbes and Aquatic Organism Disease Control Laboratory at the Department of Aquatic Biomedical Sciences, Jeju National University. Injected fish were distributed into fifteen 60 L plastic tanks and their behavior and mortality were monitored and recorded for 15 days.

Statistical analysis

All experimental diets were assigned using a completely randomized design. Data were analyzed by one-way analysis of variance (ANOVA) in SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). When ANOVA identified differences among groups, the differences in mean values were compared using Tukey's HSD test at the 5% level of significance (P <0.05). Data are presented as mean ± SD. Percentage data were arcsine transformed before analysis.

3. Results

Growth performance feed utilization and survival of fish fed the five experimental diets are shown in Table 2.3. Final body weight (FBW) and weight gain (WG) were significantly

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higher in fish fed HFM diet and significantly lower in fed fish LFM diet compared to fish fed protein hydrolysate products. Feed intake (FI) was significantly higher in fish fed HFM and TuH diets while the lowest feed intake was observed in SalH group. Feed conversion ratio (FCR) was significantly increased in fish fed LFM and HFM diets compared to the fish fed other three diets. However, the lowest FCR was shown by fish fed SalH diet. Protein efficiency ratio (PER) was significantly higher in fish fed SalH diet and lower in fish fed LFM diet. Also, fish in other tree diet had significantly higher PER compared to LFM group. Survival of fish was not significantly affected by dietary treatments.

Apparent digestibility coefficients of dry matter protein and amino acid of experimental diets are shown in Table 2.4 and 2.5. Dry matter and protein digestibility were significantly higher in HFM, SalH and TH diets compared to the LFM diet. Also, amino acid digestibility of HFM, SalH and TH diets were higher compared to LFM and TuH diets.

Hematocrit, hemoglobin and Total cholesterol level in plasma were significantly lower in fish fed LFM diet compared to the other experimental groups. No significant differences were observed in glucose and total protein levels (Table 2.6).

Innate immune responses of red seabream were significantly improved by dietary supplementation of MPHs (Table 2.7). MPO and catalase activities of HFM group were significantly increased compared to LFM group. SOD, GPx and anntiprotease activities of fish fed HFM and protein hydrolysates supplemented diets were significantly higher than LFM group. Antiprotease activity of fish fed SalH, TH and TuH diets were significantly improved compared to LFM groups. Ig level of fish fed HFM and SalH diets was significant increased compared to LFM group. Also, lysozyme activity of fish was significantly boosted by feeding TuH diet and HFM diet than LFM group. NBT activity was not significantly

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affected by dietary treatments.

Body proximate composition of red seabream viscera and eviscerated carcass composition are shown in table 2.8 and 2.9 respectively. Significantly higher carcass lipid level was observed in TH and TuH groups than HFM and LFM groups. Visceral protein amount is significantly higher in fish fed all other experimental diets than the LFM group. However, highest visceral protein level was observed in HFM group. Visceral lipid level was significant in LFM group compared to fish fed other four diets.

Morphometric parameters of fish are shown in table 2.10. GC and ID were significantly higher in fish fed HFM, SalH and TuH diet compared to LFM group. VL, EH and CF were significantly higher in fish fed HFM, SalH, TH and TuH diets compared to LFM group while the highest values were shown by HFM group. However, HSI and VSI were not significantly affected by dietary treatments.

Liver lipid level of fish was not significantly affected by experimental diets although LFM group contained numerically highest lipid level in liver. MDA amount in fish muscle was significantly decreased in HFM and fish fed MPH diets compared to LFM group. Significantly higher TAC amount in fish muscle was observed in fish fed HFM and protein hydrolysate supplemented diets (Table 2.11). Also, muscle water retention rate was not significantly affected by dietary treatments.

Significantly higher relative expression levels of liver IGF-1 mRNA was observed in fish fed HFM and MPH diets compared to LFM group (Figure 2.1). At the end of the challenge test, as shown in Figure 2.2, Survival rates were higher in HFM and MPH groups and the lowest survival rate was shown by LFM group.

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Table 2. 3. Growth performance and feed utilization of red seabream fed the five experimental diets for 12 weeks.

	IBW[1] (g)	FBW[2] (g)	WG[3] (%)	FI[4]	FCR[5]	PER[6]	Survival (%)
HFM	13.8±0.07	92.0±0.4[a]	557±6[a]	98.4±1.05[a]	1.26±0.01[bc]	1.65±0.01[ab]	97.5±2.5
LFM	13.8±0.16	73.8±1.3[c]	434±15[c]	91.5±1.95[bc]	1.52±0.02[a]	1.38±0.01[c]	95.8±1.4
SalH	13.9±0.24	86.6±2.2[b]	525±6[b]	90.6±1.35[c]	1.24±0.05[c]	1.69±0.07[a]	97.5±2.5
TH	13.7±0.03	86.8±2.9[b]	531±23[ab]	95.5±2.03[ab]	1.31±0.07[bc]	1.61±0.08[ab]	983±1.4
TuH	13.8±0.22	86.6±1.4[b]	527±15[b]	98.9±0.98[a]	1.36±0.02[b]	1.53±0.02[b]	98.3±1.4

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Initial body weight

[2]Final body weight

[3]Weight gain = [(final body weight - initial body weight)/ initial body weight] x 100

[4]Feed intake (g/fish) = dry feed consumed (g)/fish

[5]Feed conversion ratio = dry feed fed/wet weight gain

[6]Protein efficiency ratio = fish weight gain (g)/protein

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Table 2. 4. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for red seabream.

	ADCd[1] (%)	ADCp[2] (%)
HFM	77.0±1.0[a]	86.9±0.6[b]
LFM	69.3±2.7[b]	81.2±1.7[c]
SalH	74.6±0.7[b]	92.6±0.2[a]
TH	75.7±1.8[b]	86.9±1.0[b]
TuH	73.9±2.1[ab]	93.8±0.5[a]

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different (P < 0.05).

[1] Apparent digestibility coefficient of dry matter

[2] Apparent digestibility coefficient of protein

Table 2. 5. Apparent digestibility coefficients (%, ADC) for amino acid of the experimental diets for red seabream.

	Experimental diets				
	HFM	LFM	SalH	TH	TuH
Aspartic Acid	86.0±0.63	81.5±1.27	92.3±0.10	88.1±0.99	93.7±1.11
Threonine	85.4±1.05	78.3±1.41	90.9±0.26	85.8±1.30	92.0±1.49
Serin	86.0±1.46	80.7±1.46	91.8±0.57	87.0±0.90	93.0±1.32
Glutamic Acid	86.9±1.03	83.4±1.75	93.7±0.45	89.3±0.82	94.8±0.92
Proline	87.0±0.88	81.3±2.27	92.1±0.18	82.7±2.11	92.3±0.38
Glycine	85.9±0.68	78.9±1.06	89.7±0.71	88.8±0.81	92.2±0.70
Alanine	84.5±1.04	74.5±2.35	89.2±0.72	80.6±1.48	89.2±1.74
Valine	84.8±0.86	78.7±1.70	91.1±0.18	85.0±1.33	92.1±1.50
Isoleucine	84.3±0.89	80.3±1.63	91.5±0.14	85.2±1.29	92.7±1.49
Leucine	84.3±1.64	77.2±2.59	90.9±0.72	80.8±1.45	90.5±1.86
Tyrosine	84.3±1.65	79.1±2.74	91.7±1.32	83.3±0.20	94.0±2.71
Phenylalanine	84.5±1.10	79.5±1.88	91.5±0.27	85.3±1.16	92.7±1.54
Histidine	88.2±0.71	80.1±0.59	90.3±0.56	88.7±0.99	91.9±0.75
Lysine	88.5±0.31	86.3±0.64	95.1±0.15	88.7±1.06	94.4±1.18
Arginine	88.1±0.13	84.7±1.25	94.4±0.15	90.2±0.62	95.5±0.94

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different (P < 0.05).

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Table 2. 6. Hematological parameters of red seabream fed the five experimental diets for 12 weeks.

	Ht[1]	Hb[2]	Glucose[3]	Total pro[4]	Total chol[5]
HFM	34.1±1.26[a]	6.76±0.44[a]	58.6±1.8	1.94±0.07[a]	224±12
LFM	28.0±1.33[b]	5.71±0.21[b]	58.4±1.7	1.62±0.09[b]	221±15
SalH	35.9±1.17[ab]	6.67±0.22[ab]	58.2±1.1	1.84±0.04[ab]	219±02
TH	35.1±3.47[a]	6.9±0.23[a]	56.3±2.0	1.80±0.07[ab]	217±13
TuH	34.8±1.35[a]	6.47±0.4[ab]	57.7±3.1	1.85±0.05[a]	216±16

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Hematocrit (%)

[2]Hemoglobin (g dL^{-1})

[3]Glucose (mg dL^{-1})

[4]Total protein (g dL^{-1})

[5]Total cholesterol (mg dL^{-1})

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Table 2. 7. Non-specific immune response of red seabream fed the five experimental diets for 12 weeks.

	NBT[1]	MPO[2]	SOD[3]	Ig[4]	Lysoz[5]	GPx[6]	Antipro[7]	Catalase[8]
HFM	0.97±0.11	1.67±0.11[a]	70.0±0.9[a]	22.1±2.0[a]	5.4±0.1[a]	98.1±2.8[a]	17.3±1.0[a]	1.42±0.05[a]
LFM	0.83±0.07	1.35±0.09[b]	57.2±2.3[b]	14.9±0.4[b]	3.7±0.7[b]	73.9±4.1[b]	13.6±0.3[b]	1.32±0.02[b]
SalH	0.88±0.14	1.46±0.16[ab]	69.7±3.4[a]	20.4±2.8[a]	4.9±0.6[ab]	94.6±6.3[a]	16.0±0.1[a]	1.36±0.02[ab]
TH	0.96±0.06	1.47±0.08[ab]	67.5±2.9[a]	20.2±2.4[ab]	4.7±0.1[ab]	102±5.4[a]	16.0±0.6[a]	1.38±0.02[ab]
TuH	0.96±0.07	1.43±0.10[ab]	69.5±1.3[a]	19.5±1.6[ab]	5.5±0.5[a]	92.2±6.7[a]	16.8±0.4[a]	1.38±0.04[ab]

Values are mean of triplicate groups and presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Nitro blue tetrazolium activity

[2]Myeloperoxidase level

[3]Superoxide dismutase (% inhibition)

[4]Total immunoglobulin (mg mL^{-1})

[5]Lysozyme activity (μg mL^{-1})

[6]Glutathione peroxidase activity (mU ml^{-1})

[7]Antiprotease (% inhibition)

[8]Catalase activity (mU ml^{-1})

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Table 2. 8. Proximate composition of red seabream without viscera, fed the five experimental diets for 12 weeks.

	Dry matter	Protein	Lipid	Ash
HFM	41.8±3.0	21.3±1.2	13.9±0.9[a]	7.2±0.1
LFM	40.3±5.0	19.3±1.2	14.1±1.3[a]	6.9±0.13
SalH	46.9±6.5	20.8±1.3	17.0±1.9[ab]	6.8±0.32
TH	48.2±9.0	20.3±0.4	18.5±1.0[b]	6.6±0.73
TuH	48.1±7.3	21.0±1.1	18.6±3.0[b]	7.5±0.25

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

Table 2. 9. Visceral composition of red seabream fed the five experimental diets for 12 weeks.

	Dry matter	Protein	Lipid	Ash
HFM	55.2±7.0	13.1±0.8	26.9±1.5	1.03±0.11
LFM	52.9±2.7	7.8±1.1	34.7±3.0	1.17±0.12
SalH	49.9±5.2	10.9±0.7	22.4±3.3	1.20±0.08
TH	47.9±6.9	10.8±0.3	24.0±2.2	1.35±0.26
TuH	50.2±0.3	10.9±0.5	22.3±2.9	1.19±0.10

Values are mean of triplicate groups and presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$).

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Table 2. 10. Morphometric and biometric parameters of red seabream intestine fed the five experimental diets for 12 weeks.

	GC[1]	ID[2]	VL[3]	EH[4]	CF[5]	HSI[6]	VSI[7]
HFM	848±49[a]	2678±300[a]	1275±29[a]	50.4±2.1[a]	2.29±0.06[a]	2.27±0.11	4.43±0.70
LFM	538±75[b]	1831±92[b]	845±27[c]	35.3±1.5[c]	1.94±0.02[c]	1.83±0.03	5.81±0.77
SalH	783±37[a]	2458±204[a]	1187±78[ab]	47.5±0.4[ab]	2.15±0.02[ab]	2.18±0.1	5.37±0.40
TH	709±112[ab]	2226±133[ab]	1118±62[b]	43.8±2.9[b]	2.12±0.07[b]	2.38±0.37	5.54±0.41
TuH	769±79[a]	2425±46[a]	1186±26[ab]	47.6±2.9[ab]	2.15±0.07[ab]	2.41±0.30	5.42±0.25

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Goblet Cell count

[2]Intestinal Diameter (µm)

[3]Villi Length (µm)

[4]Enterocyte Height (µm)

[5]Conditional factor = (Fish weight/ Fish length^3)*100 (gcm^{-3})

[6]Hepatosomatic index= (Liver weight/ Fish weight)*100

[7]Viscerosomatic index= (Viscera weight/ Fish weight)*100

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Table 2. 11. Liver lipid and muscle immunological parameters of red seabream fed the five experimental diets for 12 weeks.

	Liver lipid	MDA[1]	MWR[2]	TAC[3]
HFM	17.4±2.5	10.0±0.5[c]	71.4±1.8	2.56±0.11[a]
LFM	24.1±3.2	14.1±0.6[a]	71.2±2.5	2.14±0.07[b]
SalH	19.3±4.6	11.3±0.5[bc]	69.0±5.9	2.54±0.13[a]
TH	20.4±4.5	11.4±0.3[b]	69.1±2.7	2.56±0.17[a]
TuH	21.9±5.2	11.7±0.6[b]	72.7±5.0	2.72±0.16[a]

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Muscle lipid peroxidation (mg/Kg)

[2]Muscle water retention (%)

[3]Muscle total antioxidant capacity (mM)

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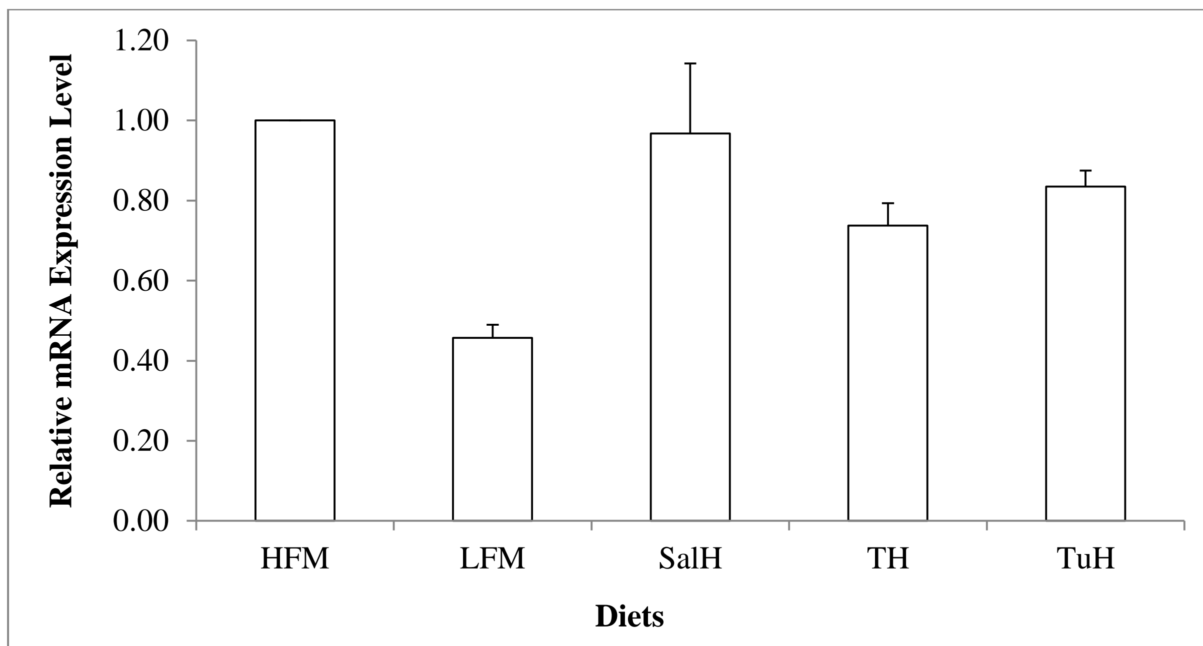

Figure 2. 1. Liver insulin-like growth factors I mRNA expression for each diet group expressed as a ratio to HFM diet values. Data are presented as mean ± SD from three replicate tanks. Different letters above the bars denote significant differences between diet groups at the $P < 0.05$ level.

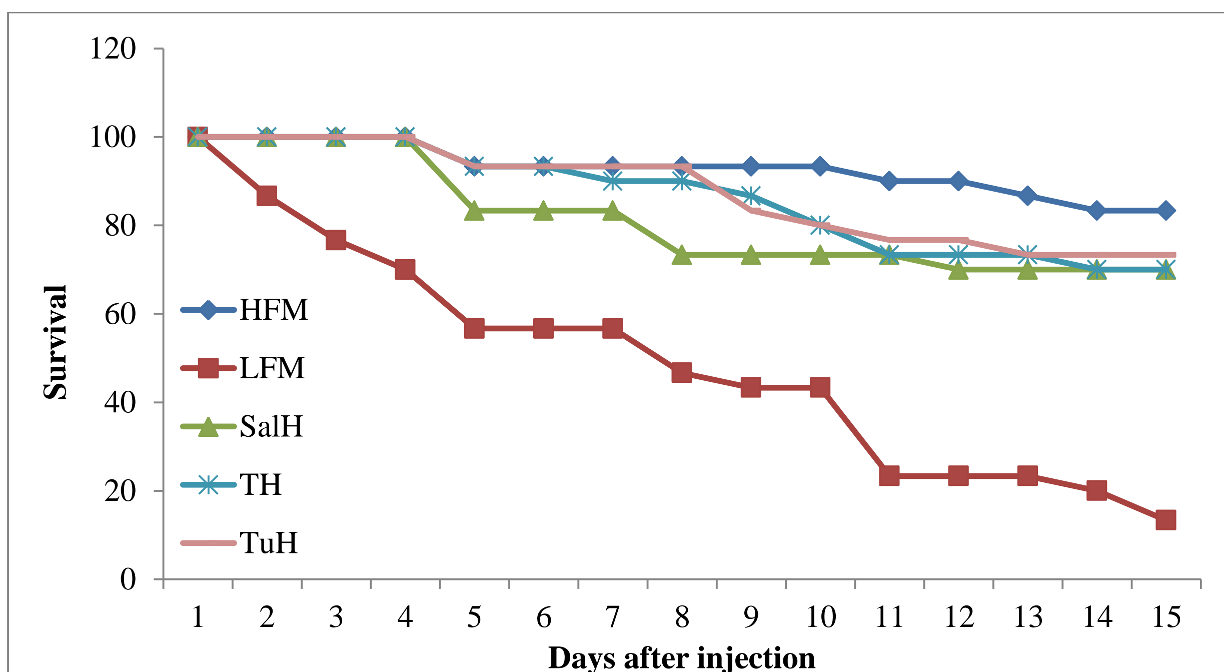

Figure 2. 2. Survival rate of red seabream fed five experimental diets for 12 weeks after challenge with *E. tarda.*

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4. Discussion

Growth performance of red seabream was significantly higher in fish fed HFM diet compared to the fish fed other experimental diets. Hydrolysate supplementation significantly improved the growth performance of fish compared to the fish fed LFM diet. Therefore, these observations indicate that the inclusion of MPHs to a LFM diet containing 13.5% FM is beneficial to improve growth performance of fish. However, in the case of growth, inclusion of 5% SalH or TH was unable to restore the effects of HFM which contain 36.8% FM. Similarly, when the same LFM diet was coated with 2% TuH, growth performance was significantly improved but the HFM effects was not restored in the fish after 12 weeks of the feeding trial. However, feed utilization results observed in this study revealed that feed utilization of HFM group can be restored in LFM diet by supplementing or coating MPH. In a previous study, we replaced 58% of FM with MPH and SPC from a HFM diet containing 50% FM, and found that HFM effects were restored in terms of growth performance and feed utilization of red seabream after 13 weeks of feeding trial (Khoshravi et al., 2015). Also, we restored the HFM effects in a LFM diet after replacing approximately 56% of FM with SPC and MPH in olive flounder diet (Khosravi et al., 2017). In that study, FM levels were 55% in HFM diet and 24% in MPH diets. Therefore, a possible reason for the present results might be the lower FM level in MPH diets (13.5%) compared to the previous studies. Kader et al. (2012) restored HFM effect in growth performance of red seabream by completely replacing FM with dehulled soybean meal, 10% fish solubles, 5% krill meal and 5% squid meal. Therefore, it can be assumed that FM could be replaced with a mixture of other animal protein sources without compromising growth performance of fish. In the study conducted by Goto et al. (2008), HFM effects were not restored on red seabream by completely replacement of FM with 20% soybean meal, 20% corn gluten meal, 20% chicken meal and 5%

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krill meal. However, when the diet contains 15% FM instead of chicken meal, the growth of fish was comparable to HFM group. Kissil et al. (2001) did not restore HFM effects on growth performance of gilthead seabream by replacing 30% of FM with SPC as the sole FM replacer. These evidences indicate that it is difficult to restore HFM effects for FM replacement with single plant or animal protein source. In this study, SPC was the sole FM replacer and FM level in LFM diet was reduced to13.5%. Growth performance might not be restored even after supplementing or coating MPH to the LFM diet. However, the feed utilization was improved by supplementing or coating hydrolysates to the LFM diet elucidating that the efficiency of feed can be enhanced by MPHs.

The protein digestibility of HFM, SalH, TH and TuH diets were significantly higher compared to the LFM diet. SalH and TuH diet showed even better protein digestibility compared to the HFM diet. These results elucidate that the diet digestibility can be improved by incorporating or coating MPH to high plant protein diets. Similar results were observed in red seabream and olive flounder diets in our previous studies (Khosravi et al., 2015, 2017). Amino acids in MPH can be more easily absorbed than those in plant protein sources (Hevroy et al., 2005). Similarly, in this study, the digestibility of amino acid was increased in MPH diets compared to LFM group (Table 2.5). Therefore, digestibility of protein might be improved due to higher absorption capability of MPH protein. The lowest FCR and the highest PER might also be resulted due to higher digestibility of nutrient compared to HFM and LFM groups.

Non-specific immune responses of red seabream were significantly improved by inclusion or coating MPH to the LFM diet. Especially, SOD, GPx and antiprotease activities of fish fed MPH diets were significantly higher than the LFM group and comparable to fish

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fed HFM diet. Some immune parameters were significantly enhanced by MPH supplementation in our previous studies (Khosravi et al., 2015, 2017). The same trend was observed in survival rate of fish after the challenge experiment. It seems that the improved immune parameters increased the survival rates after the *E.tarda* challenge experiment. Salmon hydrolysates contain antioxidant peptides which were identified to have radical scavenging ability (Ahn et al., 2014). Also, during the hydrolysis, salmon byproducts produce antihypertensive and anti-inflammatory peptides (Ahn et al., 2012, 2015). In the same way, immunomodulatory peptides were identified in TH and TuH (Je et al., 2007; Raghavan et al., 2008a, 2008b, 2009; Je et al., 2009; Hsu et al., 2010). Therefore, presence of these peptides might be a reason for the improved immunity in fish fed MPH diets. Immune responses of large yellow croaker (*Pseudosciaena crocea*) were significantly enhanced by dietary pollock (*Theragra chalcogramma*) tissue hydrolysates (Tang et al., 2008). Increased immunity of fish has been reported after feeding MPH supplemented diets (Liang et al., 2006; Gisbert et al., 2018) indicating that the immunomodulatory properties were associated with MPHs. Further studies are required to specify types and methods of MPH influence on fish immunity.

Morphometric and biometric parameters of red seabream intestine were significantly improved by dietary MPH supplementation and HFM effects were restored in LFM diets. These results were in the line with previously published results (Koshravi et al., 2015). However in this study, FM level in LFM diets were the lowest compared to previous studies. Caballero et al. (2003) observed that lipid droplet accumulation in enterocytes of gilthead seabream (*Sparus aurata*) was affected by the type of oil used in diet. Cerezuela et al. (2012) reported that intestine morphology of gilthead seabream can be changed by dietary probiotics and microalgae. Ferrara et al. (2015) observed inflammations and malfunctions in

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sharpsnout seabream (*Diplodus puntazzo*) gut when they fed diets containing high levels of soybean meal. Ostaszewska and Dabrowski. (2005) reported that high plant protein in fish diets can affected to gut morphology and caused disorders in digestion and absorption. According to these evidences,, reduced gut morphometric parameters observed in the fish fed LFM diet might be attributed to the presence of high SPC level in the present study. However, inclusion of MPH diets restored the HFM effects in the LFM diet.

In conclusion, these results indicate that reduced feed utilization and immune responses of red seabream by LFM diet, which was prepared by substituting 63.3% of FM from a HFM diet with SPC, can be recovered by supplementing MPHs. Further studies are required to evaluate the effects of hydrolysates supplementation by increasing the FM level of LFM diets for red seabream.

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Chapter III

Effects of shrimp protein hydrolysates and/or tilapia protein hydrolysates supplemented low fish meal diets on growth, feed utilization, innate immunity, intestine morphology, diet digestibility and disease resistance of red seabream (*Pagrus major*).

1. Introduction

Aquaculture provides approximately half of all fish for human consumption as a growing industry during last few decades (FAO 2018). In the case of fish farming, sufficient well balanced feed is the major factor that can improve production. The demand of FM is growing by 8% annually as a major animal protein source in fish and shrimp feeds (FAO, 2018). FM replacement is an important strategy to minimize the cost of production while increasing yield.

MPHs are rich source of bioactive compounds such as amino acids and peptides which can be easily absorbed by animals (Wisuthiphaet et al., 2015). In the case of aquaculture, MPH can be used as a substitute for FM as a usable source of animal proteins. Previous studies proved that MPH is possible source to provide well balanced nutrients to fish and shrimp fed low FM diet species (Khosravi et al., 2015, 2017). Also, innate immunity, growth performance, feed utilization and biochemical parameters of fish and shrimp found to be increased by feeding MPH supplemented LFM diets.

Partial replacement of FM with MPH in fish diets were evaluated in previous studies and positive effects were observed on growth, feed utilization, innate immunity, intestine histology, digestibility, IGF-1 mRNA expression and disease resistance against pathogens for

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olive flounder and red seabream (Bui et al., 2013; Khosravi et al., 2015, 2017).

Present study was conducted to examine the effects of shrimp and/or tilapia hydrolysates on growth performance, feed utilization, intestinal morphology, digestibility, innate immunity, hematological parameters, and disease resistance against pathogens of red seabream.

2. Materials and Methods

Experimental diets

Five experimental diets were formulated to be isonitrogenous (44.9% crude protein) and isocaloric (18 kJ g^{-1}) (Table 3.1) by inclusion of Protein hydrolysates those were all provided by AQUATIV (Aquaculture Division of DIANA, Member of SYMRISE Group), Elven, France. A FM-based diet (37%) was regarded as a high FM diet (HFM) and a diet contained SPC, as a substitute for FM, at replacement level of 50% was considered as low FM diet (LFM). Three other experimental diets (TH, TH/SH and SH) were prepared by dietary inclusion of Tilapia hydrolysate, mixture of tilapia and shrimp hydrolysates (1:1) or shrimp hydrolysate in low FM based diet at 5% inclusion level. All diets were coated with 4% of fish oil.

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Table 3. 1. Formulation and proximate composition of the experimental diets for red seabream (%, dry matter basis)

Ingredients	Experimental diets				
	HFM	LFM	TH	TH/SH	SH
FM FAQ65	37.0	18.5	18.5	18.5	18.5
Tilapia hydrolysate			4.85	2.43	
Shrimp hydrolysate				2.38	4.75
Soy protein concentrate (SPC)	9.90	24.3	19.8	20.2	20.5
Corn gluten meal	12.0	12.0	12.0	12.0	12.0
Wheat flour	28.7	28.4	28.65	28.4	28.5
Fish oil	4.93	6.0	6.0	5.9	5.7
Soybean oil	4.00	4.00	4.00	4.00	4.00
Mineral Mix1	1.00	1.00	1.00	1.00	1.00
Vitamin Mix2	1.00	1.00	1.00	1.00	1.00
Starch	0.97	1.60	1.00	1.00	0.85
Choline chloride	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.00	0.50	0.50	0.50	0.50
L-Methionine	0.00	0.20	0.20	0.20	0.20
Taurine	0.00	0.50	0.50	0.50	0.50
Di-calcium phosphate	0.00	1.50	1.50	1.50	1.50
Proximate composition					
Crude protein	47.4	46.2	46.8	47.1	47.3
Crude lipid	14.0	13.1	13.0	13.1	12.9
Crude ash	7.80	7.90	7.70	7.90	7.80
Moisture	8.10	7.90	7.90	7.50	7.90

[1]Mineral premix (g kg^{-1} of mixture): $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3$. $6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[2] Vitamin premix (g kg^{-1} of mixture): L-ascorbic acid, 121.2; DL-α tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-$_D$-pantothenate, 12.7; myo-inositol, 181.8; $_D$-biotin, 0.27; folic acid, 0.68; p-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

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Table 3. 2. Amino acid composition (% in sample) of the experimental diets for red seabream

	Experimental diets				
	HFM	LFM	TH	TH/SH	SH
Aspartic Acid	3.53	4.04	3.94	3.91	3.72
Threonine	1.53	1.60	1.60	1.59	1.53
Serin	1.65	1.89	1.82	1.85	1.80
Glutamic Acid	7.27	8.60	8.19	8.06	7.93
Proline	2.45	2.81	2.76	2.77	2.51
Glycine	2.15	2.02	2.34	2.20	1.96
Alanine	2.44	2.48	2.52	2.45	2.36
Valine	2.09	2.24	2.23	2.18	2.07
Isoleucine	1.78	2.00	1.97	2.00	1.84
Leucine	3.60	4.06	3.86	3.87	3.71
Tyrosine	1.10	1.27	1.20	1.12	1.13
Phenylalanine	1.87	2.21	2.10	2.08	2.03
Histidine	1.31	1.43	1.38	1.40	1.35
Lysine	2.40	2.79	2.92	2.85	2.67
Arginine	2.25	2.51	2.50	2.45	2.24

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Table 3. 3. The molecular weight distribution, proximate and essential amino acids composition of protein hydrolysates (from product technical data sheets).

	SH[1]	TH[1]
Molecular weight (Da, %)		
>30,000	<0.1	<0.1
20,000–30,000	<0.1	<1
10,000–20,000	<0.1	3.0
5000–10,000	<0.5	4.0
1000–5000	8	18
500–1000	9	14
<500	83	60
Proximate composition (dry matter, %)		
Dry matter	96.0	95.0
Protein	67.6	74.9
Lipid	11.3	16.2
Ash	10.7	5.1
Soluble nitrogen (% N)	91.1	91.5
Taurine	1.25	0.79
Essential amino acids (products, %)		
Arg	4.15	4.06
His	1.55	1.36
Ile	2.71	2.45
Leu	4.28	4.38
Lys	4.13	4.23
Met	1.26	1.58
Phe	2.95	2.42
Thr	2.62	2.77
Val	3.34	2.94

[1]SH = shrimp hydrolysate; TH = tilapia hydrolysate

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Fish and feeding trial

Juvenile red seabream were transported from a private hatchery to the Marine and environmental research institute, Jeju National University, Jeju, Korea. All fish were acclimatized to experimental conditions and facilities for two weeks while feeding a commercial diet. Six hundred fish (Initial mean body weight, 9.67±0.07g) were randomly distributed into fifteen polyvinyl circular tanks (40 fish / tank). Each tank was randomly assigned to one of the three replicates of five dietary treatments and supplied with filtered sea water at a flow rate of 3 L min^{-1} and aerated with sandstones to maintain sufficient dissolved oxygen. Fish were fed the experimental diets twice a day (09.00h and 18.00h) until satiation for 17 weeks. Growth checking was conducted at three weeks interval. Feeding was stopped 24 hour prior to weighting to minimize stress. Uneaten feed was collected 30 min after feeding and reweighed to determine feed intake.

Sample collection

At the end of the feeding trial, all the fish from each tank collectively weighted to obtain total biomass. Four fish from each tank were randomly selected, anesthetized with 2-Phenoxy ethanol solution (200 ppm) and blood was collected from caudal vein with heparinized syringes for determine hematocrit and hemoglobin. Then plasma were separated by centrifugation at 5000×g for 10 min and stored at -70 ◦C until analyze total immunoglobulin (Ig), antiprotease activity and biochemical parameters. Another set of blood samples were taken from the caudal vein of four fish from each tank using non heparinized syringes, allowed clotting at room temperature for 30 min and the serum was separated by centrifugation for 10 min at 5000×g and stored at -70 ◦C for analysis of innate immune response parameters.

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Estimation of apparent digestibility coefficients

Chromic oxide (Cr_2O_3) (Sigma-Aldrich, St. Louis, USA) was included in the diets as an inert indicator at a concentration of 1.0% for estimation of apparent digestibility coefficient of the experimental diets according to the method described in our previously published paper (Khosravi et al., 2015). New sets of red seabreams were used for the digestibility test in a fecal collection system (Guelph system) tanks at a density of 50 fish per tank.

Analyses

Proximate composition analysis

Analysis of moisture and ash content were performed by the standard procedures (AOAC, 1995). Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FOSS, Sweden) and crude lipid was determined using Soxhlet Extraction System C-SH6 (Korea).

Hematological parameters

Hematocrit was determined by microhematocrit technique (Brown, 1980). Hemoglobin and plasma levels of glucose, total protein and cholesterol were determined by an automated blood analyzer (SLIM, SEAC Inc, Florence, Italy).

Immunological assays

The oxidative radical production by phagocytes during respiratory burst was measured through NBT assay described by Anderson and Siwicki (1995). Plasma immunoglobulin (Ig) levels were determined according to the method described by Siwicki and Anderson (1993). A turbidometric assay was used for determination of serum lysozyme

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level through the method described by Hultmark (1980) with slight modifications. Serum myeloperoxidase (MPO) activity was measured according to Quade and Roth (1997). Serum superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase using a SOD Assay Kit (Sigma, 19160) according to the manufacturer's instructions. The plasma anti-protease activity was measured according to the method described by Ellis (1990), with slight modifications (Magnadóttir et al., 1999). GPx activity was assayed using kit (Biovision, Inc. California, USA). Catalase activity was assayed using kit (Biovision, Inc. California, USA). Total antioxidant capacity (TAC) was determined using a commercial kit (Antioxidant assay kit, Sigma; CS0790). Muscle malondialdehyde (MDA) levels were determined spectrophotometrically using Lipid Peroxidation (MDA) Assay Kit (Biovision, Inc., Milpitas, CA, USA).

Intestinal morphology

Another set of two fish per tank collected and the whole intestine removed and sampled for its histology. Body weight, liver weight, viscera weight intestine length and fish length were measured and recorded for calculation of CF, HSI and VSI. The intestine samples were fixed in Bouins solution, dehydrated in graded series of ethanol, embedded in paraffin and then sectioned in 5μm sagittal serial sections.

Slides were stained with Harris hematoxylin and 0.5% eosin for general histological observation and the Alcian blue (AB) at pH 2.5 and periodic acid Schiff (PAS) for observation of the mucus secreting goblet cells. The morphometric measurements of villus length (VL), intestine diameter (ID) and enterocyte height (EH) were made, using the ImageJ 1.44 analysis software.

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Challenge test

At the end of the feeding trial, ten fish from each tank (30 fish per treatment) were intraperitoneally injected with *Edwardsiella tarda* (ATCC 15947, Korea Collection for Type Cultures) suspension (1×10^5 CFU ml^{-1}). *E. tarda* was provided by the Marine Applied Microbes and Aquatic Organism Disease Control Laboratory at the Department of Aquatic Biomedical Sciences, Jeju National University. Injected fish were distributed into fifteen 60 L plastic tanks and their behavior and mortality were monitored and recorded for 22 days.

Statistical analysis

All experimental diets were assigned using a completely randomized design. Data were analyzed by one-way analysis of variance (ANOVA) in SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). When ANOVA identified differences among groups, the differences in mean values were compared using Tukey's HSD test at the 5% level of significance (P <0.05). Data are presented as mean ± SD. Percentage data were arcsine transformed before analysis.

3. Results

Growth performance, feed utilization and survival of fish fed the experimental diets are shown in Table 3.4. FBW of SH diet fed fish groups were significantly higher compared to HFM and LFM diet and fish fed TH and TH/SH diets were comparable to HFM group while significantly lower body FBW was shown in LFM group. Also, WG of fish fed HFM and hydrolysate supplemented diets were significant to LFM group. Feed intake (FI) of red seabreams was significantly increased by HFM and hydrolysate supplemented diets compared to LFM diet. However, the highest feed consumption was shown by HFM group. FCR and PER were significantly improved by hydrolysates supplemented diet compared to

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HFM and LFM groups. Survivals of fish were not affected by any dietary treatments.

Apparent digestibility coefficient of protein, dry matter and amino acids in experimental diets are shown in Table 3.5 and 3.6. Protein digestibility of fish fed SH was significantly higher compared to other dietary treatment while LFM group showed the lowest protein digestibility. However, DM digestibility was not significantly affected by the dietary treatments. Amino acid digestibility was significantly increased by the test diets compared to LFM diet.

Blood hematocrit percentage was significantly higher in fish fed TH and TH/SH diets compared to LFM group. Also, hemoglobin levels of blood was significantly increased by dietary TH supplementation compared to fish fed LFM diet. Glucose, triglyceride, cholesterol and total protein levels of plasma were not significantly affected by the diets (Table 3.7). Plasma LDL was significantly lower in fish fed HFM and SH diets compared to LFM group. Muscle malondialdehyde level was significantly higher in fish fed LFM diet compared to TH/SH group. However, TAC of red seabreams was not significantly affected by the dietary treatments (Table 3.8).

Innate immune responses of red seabream were significantly improved by dietary supplementation of SH and/or TH (Table 3.9). Catalase activity of RSB was significantly increased by TH/SH diet compared to fish fed LFM groups. Serum lysozyme activity of HP1 group was significantly higher than LFM group. NBT activity was significantly higher in fish fed TH or SH diets compared to LFM group. SOD activity was significant in fish fed HFM, TH/SH and SH diets than the LFM group. Also, plasma Ig level was significant in HFM, TH and SH groups compared to LFM group. No significant differences were observed in antiprotease and GPx activities.

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Analyses of whole body proximate composition was not revealed significant differences in whole body, carcass or viscera by dietary treatments (Table 3.10, 3.11 and 3.12).

Morphometric parameters of fish intestine are shown in Table 3.13. Compared to LFM group, VL of fish intestines were significantly longer in fish fed HFM and hydrolysate diets while TH/SH and SH group showed the longest villi. EH was significantly higher in fish fed HFM, TH/SH or SH diets and lower in fish fed TH or LFM groups. Also, ID was significant in TH/SH groups than the other dietary treatments. However, Goblet cell counts were not significant after 17 weeks of feeding trial. Biometric parameters of red seabream intestine were no significantly affected by experimental diets (3.14).

At the end of the challenge experiment, as shown in Figure 3.1, the highest survival rates were obtained by SH group and the lowest survival rates were shown by fish fed LFM diet. Survival rates of fish fed HFM and hydrolysate supplemented diet were significant to LFM group. Specially, PBS group had no mortality during experiment period.

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Table 3. 4. Growth performance and feed utilization of red seabream fed the five experimental diets for 12 weeks.

	IBW[1] (g)	FBW[2] (g)	WG[3] (%)	FI[4]	FCR[5]	PER[6]	Survival (%)
HFM	9.2±0.18	103±5.2[b]	966±36.1[a]	140±2.05[a]	1.50±0.06[a]	1.38±0.05[b]	98.3±1.4
LFM	9.8±0.21	91.5±0.6[c]	836±25.8[b]	126±2.58[c]	1.54±0.03[a]	1.38±0.03[b]	96.7±1.4
TH	9.6±0.05	110±4.3[ab]	1043±51.2[a]	132±1.47[b]	1.32±0.06[b]	1.59±0.07[a]	98.3±1.4
TH/SH	9.6±0.19	108±1.5[ab]	1024±37.7[a]	133±0.26[b]	1.35±0.02[b]	1.54±0.02[a]	98.3±1.4
SH	9.6±0.23	112±1.7[a]	1065±36.1[a]	133±1.77[b]	1.30±0.03[b]	1.72±0.02[a]	98.3±1.4

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Initial body weight

[2]Final body weight

[3]Weight gain = [(final body weight - initial body weight)/ initial body weight] x 100

[4]Feed intake (g/fish) = dry feed consumed (g)/fish

[5]Feed conversion ratio = dry feed fed/wet weight gain

[6]Protein efficiency ratio = fish weight gain (g)/protein

Table 3. 5. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for red seabream.

	ADCp[1] (%)	ADCd[2] (%)
HFM	87.1±1.0[bc]	70.9±2.4
LFM	81.4±0.3[d]	70.8±0.5
TH	86.3±0.3[c]	73.5±0.7
TH/SH	88.3±0.7[ab]	73.2±1.7
SH	89.4±0.6[a]	73.8±1.3

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$).

[1] Apparent digestibility coefficient of protein

[2] Apparent digestibility coefficient of dry matter

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Table 3. 6. Apparent digestibility coefficients (%, ADC) for amino acid of the experimental diets for red seabream.

	HFM	LFM	TH	TH/SH	SH
Aspartic Acid	79.3±1.5	77.0±0.2	80.3±0.5	79.9±1.0	79.3±1.1
Threonine	78.9±1.5	76.1±0.5	79.9±0.5	78.7±1.2	79.0±1.1
Serin	79.6±1.0	77.3±0.8	80.1±1.1	79.4±1.3	80.7±1.4
Glutamic Acid	81.6±1.6	79.3±0.5	81.9±0.1	81.1±1.5	82.0±0.8
Proline	81.5±1.7	79.6±0.3	81.5±1.6	82.8±2.2	80.1±1.0
Glycine	84.2±1.2	79.7±0.4	85.7±0.5	84.2±0.9	82.7±0.8
Alanine	79.5±2.0	76.5±0.6	79.9±0.5	77.9±1.1	79.5±1.4
Valine	78.8±2.1	76.0±0.7	79.5±1.0	78.5±1.3	78.1±1.2
Isoleucine	77.8±2.1	75.5±1.1	79.0±0.8	78.6±1.0	77.6±1.0
Leucine	78.0±2.3	76.0±0.6	77.5±1.0	76.6±1.4	77.4±1.1
Tyrosine	79.4±0.6	76.1±3.3	77.3±4.1	72.6±1.8	78.8±3.0
Phenylalanine	78.3±2.0	76.3±0.7	78.8±0.4	77.4±0.9	78.2±1.0
Histidine	79.2±2.0	77.7±1.7	80.9±2.2	81.6±0.7	77.9±3.5
Lysine	81.4±0.9	81.8±1.5	85.7±0.4	85.1±1.3	84.7±1.8
Arginine	81.7±2.2	79.3±0.9	82.4±0.2	82.2±2.1	81.4±2.1

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different (P < 0.05).

Table 3. 7**.** Hematological parameters of red seabream fed the five experimental diets for 17 weeks.

	Ht[1]	Hb[2]	Glucose[3]	Trigly[4]	Cholel[5]	Total prot[6]
HFM	44.9±1.0^{ab}	7.11±0.4^{ab}	22.7±2.6	156±32	118±8	1.95±0.15
LFM	41.0±2.3^{b}	6.51±0.0^{b}	24.3±1.5	111±9	107±14	1.91±0.45
TH	45.9±2.6^{a}	7.56±0.4^{a}	25.1±5.3	132±30	114±14	1.99±0.17
TH/SH	45.9±1.3^{a}	7.04±0.2^{ab}	21.7±3.1	153±12	125±8	1.89±0.27
SH	44.7±0.6^{ab}	7.06±0.1^{ab}	25.3±5.5	149±18	124±4	2.29±0.27

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Hematocrit (%)

[2]Hemoglobin (g dL^{-1})

[3]Glucose (mg dL^{-1})

[4]Triglyceride (mg dL^{-1})

[5]Total cholesterol (mg dL^{-1})

[4]Total protein (g dL^{-1})

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Table 3. 8. Plasma HDL, LDL levels, muscle MDA level and TAC of red seabream fed the five experimental diets for 17 weeks.

	HDL[2]	LDL[3]	MDA[4]	TAC
HFM	72.0±3.17	58.0±2.45[bc]	8.47±0.84[ab]	2.45±0.19
LFM	69.7±1.54	62.4±0.91[a]	8.85±1.13[a]	2.33±0.07
TH	71.0±2.01	59.7±0.82[ab]	7.02±0.44[ab]	2.51±0.06
TH/SH	70.3±1.26	59.7±0.69[ab]	6.29±0.94[b]	2.51±0.11
SH	73.0±1.30	55.7±1.35[c]	6.66±1.07[ab]	2.31±0.14

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($P < 0.05$).

[1]Total protein (g dL^{-1})

[2]High-density lipoprotein (mg dL^{-1})

[3]Low-density lipoprotein (mg dL^{-1})

[4]Malondialdehyde level (mg/Kg)

Table 3. 9. Non-specific immune response of red seabream fed the five experimental diets for 17 weeks.

	Antipr[1]	Catalase[2]	GPx[3]	MPO[5]	NBT[6]	SOD[7]	Ig[8]
HFM	17.6±2.6	0.66±0.05[ab]	97.4±13	1.56±0.29	1.21±0.02[ab]	54.7±3.9[a]	20.1±0.93[a]
LFM	14.3±1.2	0.58±0.07[b]	91.1±9.9	1.31±0.09	1.15±0.03[b]	48.5±2.6[b]	16.4±1.45[b]
TH	16.5±3.4	0.74±0.10[ab]	96.0±15	1.47±0.15	1.31±0.07[a]	51.1±3.0[ab]	20.5±0.98[a]
TH/SH	19.7±3.3	0.85±0.20[a]	117.0±12	1.45±0.05	1.18±0.01[b]	54.6±1.8[a]	19.5±1.19[ab]
SH	19.7±3.4	0.73±0.14[ab]	111.0±15	1.44±0.03	1.25±0.10[a]	54.6±2.0[a]	21.5±1.59[a]

Values are mean of triplicate groups and presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1] Antiprotease (% inhibition)

[2] Catalase activity (mU ml^{-1})

[3] Glutathione peroxidase activity (mU ml^{-1})

[4] Lysozyme activity (µg mL^{-1})

[5] Myeloperoxidase level

[6] Nitro blue tetrazolium activity

[7]Superoxide dismutase (% inhibition)

[8]Total immunoglobulin (mg mL^{-1})

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Table 3. 10**.** Proximate composition of red seabream fed the five experimental diets for 17 weeks.

	Dry matter	Protein	Lipid	Ash
HFM	36.1±1.9	17.4±0.2	12.1±1.9	4.3±0.53
LFM	37.5±2.4	18.3±0.7	10.2±0.3	4.0±0.49
TH	35.8±0.9	16.1±1.5	10.1±1.5	4.7±0.97
TH/SH	37.2±0.5	18.4±0.5	11.9±1.8	4.3±0.92
SH	38.7±2.9	19.3±0.5	10.6±1.5	4.3±0.43

Values are mean of triplicate groups and presented as mean ± S.D.

Table 3. 11**.** Viscera proximate composition, liver lipid content and muscle water retention (%, wet basis) of red seabream fed the five experimental diets for 17 weeks.

	VP[1]	VL[2]	LL[3]	MWR[4]
HFM	12.3±2.56	62.5±2.86	22.4±2.16	71.1±1.79
LFM	11.1±1.55	62.5±2.51	24.7±2.02	70.5±1.50
TH	11.7±2.05	61.9±4.03	21.6±1.53	71.6±0.95
TH/SH	12.8±2.22	61.6±3.24	22.4±3.15	71.4±0.06
SH	12.2±2.48	62.9±2.02	22.1±0.43	71.1±0.79

Values are mean of triplicate groups and presented as mean ± S.D.

[1]Viscera crude protein (%)

[2]Viscera crude lipid (%)

[3]Liver crude lipid (%)

[4]Muscle water retention (%)

Table 3. 12**.** Whole-body eviscerated carcass proximate composition (%, dry matter) of red seabream fed the five experimental diets for 17 weeks.

	Protein	Moisture	Ash	Lipid
HFM	46.0±2.28	62.1±1.33	13.9±0.79	31.7±2.38
LFM	45.2±1.53	61.7±1.11	13.0±0.31	33.8±1.50
TH	45.8±2.01	63.7±4.42	12.8±1.55	32.9±2.11
TH/SH	46.3±0.92	62.7±1.06	13.7±2.99	31.5±1.05
SH	46.4±1.60	61.4±0.17	13.9±1.35	33.8±2.33

Values are mean of triplicate groups and presented as mean ± S.D.

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Table 3. 13**.** Morphometric parameters of red seabream intestine fed the five experimental diets for 12 weeks.

	VL[1]	EH[2]	ID[3]	GC[4]
HFM	1073±30[ab]	49.3±1.6[a]	2923±102[ab]	840±32
LFM	803±17[c]	35.0±2.0[b]	2688±143[b]	761±35
TH	1010±62[b]	36.2±3.9[b]	2976±201[ab]	897±31
TH/SH	1168±30[a]	52.3±2.1[a]	3062±129[a]	894±68
SH	1162±37[a]	53.3±5.6[a]	2953±33[ab]	906±92

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1] Villi Length (µm)

[2] Enterocyte Height (µm)

[3] Intestinal Diameter (µm)

[4] Goblet Cell count

[5]Conditional factor = (Fish weight/ Fish length^3)*100 (gcm^{-3})

[6]Hepatosomatic index= (Liver weight/ Fish weight)*100

[7]Viscerosomatic index= (Viscera weight/ Fish weight)*100

Table 3. 14**.** Biometric parameters of red seabream intestine fed the five experimental diets for 17 weeks.

	CF[1]	HSI[2]	VSI[3]
HFM	2.19±0.35	1.86±0.15	5.96±0.63
LFM	1.84±0.21	1.89±0.24	6.33±0.52
TH	2.16±0.21	2.25±0.23	6.05±0.63
TH/SH	1.90±0.24	2.25±0.08	6.86±0.94
SH	2.14±0.09	1.97±0.06	5.30±0.25

Values are mean of triplicate groups and presented as mean ± S.D.

[1]Conditional factor = (Fish weight/ Fish length^3) x 100

[2]Hepatosomatic index = (Liver weight/ Fish weight) x 100

[3]Viscerosomatic index = (Viscera weight/ Fish weight) x 100

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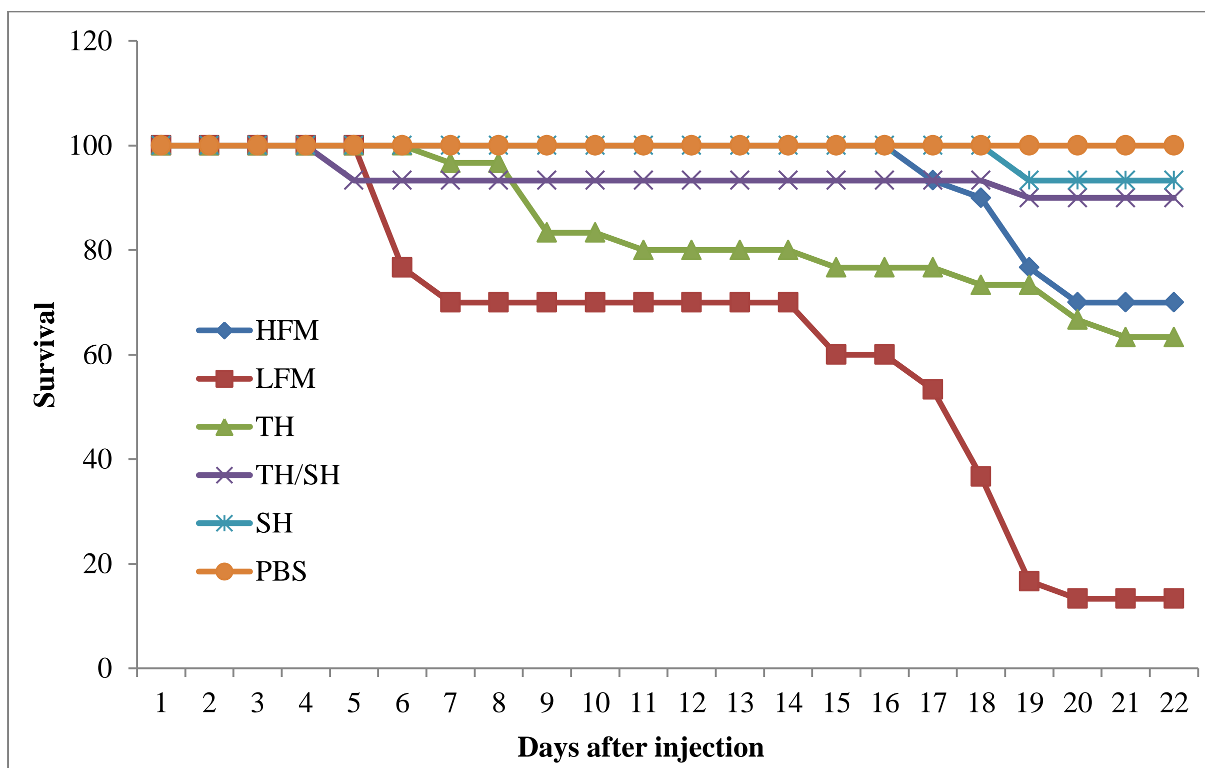

Figure 3. 1. Survival rate of red seabream fed five experimental diets for 17weeks after challenge with *E. tarda.*

4. Discussion

The results revealed that the growth and feed utilization of fish were improved by both hydrolysates restoring HFM effects. SH group showed even better FBW compared to that of the HFM group. Both TH and SH contain high levels of peptides in small molecular weight, amino acids and soluble nitrogen. Amino acids and peptides act as growth promoter in fishes (Gómez-Requeni et al., 2004; Tesser et al., 2005; Dabrowski et al., 2010; Kaushik and Seiliez, 2010). Studies conducted using different fish species revealed that low molecular weight compound promote the growth of fishes (Aksnes et al., 2006; Van Doan et al., 2014). Therefore, those compounds in SH and TH might be a reason to improve growth performance and feed utilization of fish in this study. SH contains astaxanthin which was reported to

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enhance growth performance of some fish species (Li et al., 2018). Also, SH contains higher taurine level compared to TH. It was reported by several studies that the dietary taurine enhance the growth performance of fish. A study conducted by our research group confirmed that HFM effects could be restored in red seabream by feeding a taurine incorporated LFM diet (Gunathilaka et al., 2019). Therefore, these reasons might be affected to the higher growth performance of fish fed SH diet than the HFM group.

Similar results to the present study were observed in fish fed SH and TH in our previous studies (Bui et al., 2014; Khosravi et al., 2015, 2017). Several studies reported that dietary supplementation of MPH can develop fish intestine to increase absorption surface area (Bui et al., 2014; Khosravi et al., 2015, 2017). Leduc et al. (2018) reported that SH can improve myotropic activity of fish intestine. Marchbank et al. (2009) demonstrated intestine protective effects of MPHs under damaging effects of indomethacin. The activity of digestive enzymes and gut microbiota can be enhanced by MPH (Kotzamanis et al., 2007). Ostaszewska et al. (2010) observed that diet containing peptides increase the number of cholecystokinin (CCK) positive cells and facilitate the digestion in intestine of common carp (*Cyprinus carpio*). TH and SH also contain high level of peptides compared to LFM and HFM diets. In accordance with these evidences, it is reasonable to assume that TH and SH exert an ability to develop morphology and health status of fish gut to improve digestibility of diets.

Innate immunity of red seabream was improved by dietary TH and SH supplementation in the present study. Catalase, SOD, NBT activities and Ig level were significantly increased. It is believed that the innate immune system is the most important defense mechanism of fishes as the adaptive immune system is not well functioning

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(Magnadóttir, 2006). SH and TH contain high levels of peptides by the process of hydrolysis. Peptides play an important role in the innate immune responses of fishes (Smith et al., 2000; Cuesta et al., 2007). Antimicrobial peptides were identified in MPHs by several studies (Sila et al., 2014). Peptides of MPHs involved in lymphocyte proliferation and increased the number of plaque-forming cells of mice (Yang et al., 2009; Hou et al., 2012) It was reported that mucous surfaces of fish, first barrier against pathogens, contain antimicrobial peptides along with other immune parameters to destroy pathogens (Magnadóttir, 2006). Therefore, the presence of high peptide level might be a reason for the increased immunity by SH and TH incorporated diet compared to LFM and HFM diets. According to Chalamaiah et al. (2014), protein hydrolysates produced by rohu (*Labeo rohita*) eggs stimulated humoral and cellular immune responses and increased the peritoneal macrophages as well as enhanced mucosal immunity of mice after oral administration. With the results observed in this study, it can be assumed that SH and TH shows the same performance in red seabream to enhance immune parameters. In addition, SH contain astaxanthin which is a well-known immonostimulant capable to improve immunity of fish after dietary supplementation. The enhanced immunity was proved by the results of the challenge test. Further studies are required to find possible mechanism/s of MPH by which immune responses of fish could be increased.

VL, EH and ID of red seabream intestine were significantly affected by feeding TH or SH containing diets. These three parameters indicate that the surface area of the intestine was increased by dietary supplementation of TH or SH. These observations were in the line with results of our previous studies (Bui et al., 2014; Khosravi et al., 2015, 2017). High level of plant protein in diets caused to reduce intestine surface area and enzyme activity of

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Atlantic salmon (Krogdahl et al., 2003). In the present study, similar results were observed in fish fed LFM diet and it was recovered when LFM diet was added with SH or TH. A study conducted by Bonaldo et al. (2011) observed no changes in gut morphology of juvenile turbot (*Psetta maxima*) fed a diet containing 25% LT FM and 22% soybean meal.

In conclusion, therefore, these results indicate that dietary supplementation of SH and/or TH LFM diet containing 18.5% FM, can improve the performance of red seabreams better than a HFM diet which contain 37% FM.

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Chapter IV

Effects of shrimp protein hydrolysate and krill meal supplemented low fish meal diets on growth performances, immune status and liver IGF-1 mRNA expression of red seabream

1. Introduction

FM replacement with alternative protein sources is considered as a major research area in aqua feed industry over the last few decades due to limited FM supply and higher demand. Fisheries byproducts and other marine protein sources have been used for the FM replacement studies and/or as supplements to compensate impaired growth performance in relatively low FM diets ((Plascencia-Jatomea at el., 2002; Leal at el., 2010; Koshravi et al., 2015a; Leduc at el., 2018).. As the result, advanced processing technologies have been developed to improve the quality of the byproducts or marine protein sources fish by products and marine protein sources (Dong et al., 2008; Chalamaiah et al., 2012).

Shrimp hydrolysates (SH) are produced from shrimp byproduct or processing wastes such as shrimp heads, cuticles or tail muscles. Specially, shrimp cephalothorax and exoskeleton, asthaxanthin and protein are account for 35-45% of wholebody weight as inedible wastes (Meyers 1986; Shahidi & Synowiecki, 1991). Therefore, several methods were exploited to make the byproducts edible or valuable, and hydrolysis method was recommended as a successful way to convert byproducts into micro nutrients (Nwanna, 2003; Lopez-Cervantes et al., 2006; Bueno-Solano et al., 2009; Cahu et al., 2012). The studies on SH revealed antioxidant, anti-hypertensive, antimicrobial and myotropic activities (Nii et al., 2008; Huang et al 2011; Kleekayai et al., 2015; Leduc at el., 2018). Further, trace amounts of heavy metals, such as Hg, Pb and Cd were recorded in shrimp byproducts while free amino acids content was 15% higher than the edible parts of shrimp (Heu et al., 2003). In this regard,

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SH has been evaluated in diets for fish and shrimp species, and positive effects were elucidated on growth, immunity, digestibility, palatability and health (Plascencia-Jatomea at el., 2002; Leal at el., 2010; Koshravi et al., 2015a; Koshravi et al., 2015b; Khoshravi et al., 2017; Leduc at el., 2018).

Krill are small crustaceans classified into order Euphausiacea. They are one of the primary food sources for large marine mammals and fishes (Hardy, 2008). Also, they are a rich source of marine proteins, omega 3 fatty acids, phospholipids and astaxanthine. Therefore, krill processing by-products are used to produce valuable products, such as krill meal (KM), krill hydrolysates and krill oil (Tilseth and Hostmark, 2009). It is a good palatability enhancer in addition to suitable lipid and mineral sources for fish (Goto et al., 2001; Hansen et al., 2010). Therefore, increased feed intake has been reported in fish species when diets are incorporated with KM (Yoshitomi et al., 2006; Hatlen et al., 2017). It has been proven that dietary KM supplementation can improve growth performance, feed utilization, health status, digestibility and disease resistance of fish (Gaber 2005; Hansen et al., 2010; Yan et al., 2018). KM was also included as a feed ingredient in low FM diets for red seabream (Takagi et al., 2001; Kader et al., 2012). Shimizu and Chiaki (1990) reported that feeding behavior of red seabreams was stimulated by dietary KM supplementation. Later, Kader et al. (2010) replaced a high proportion of FM with SPC and KM in red seabream diet without compromising feeding behavior, growth or health. Recently, Cho et al. (2018) tested KM as a main protein source in red seabream diet and observed that amino acids and fatty acids profiles of the fish were improved by KM.

Red seabream is widely cultured fish species in East Asian region. It was the second largest cultured fish species in Japan (Koshio, 2002) and the third one in South Korea (Kim et

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al., 2012; KOSTAT, 2017). In our previous studies, FM was successfully replaced with SH and krill hydrolysates (KH) from a HFM diet for red seabream and HFM effects were restored in diets containing high levels of plant protein (Bui et al., 2014; Khosravi et al., 2015).) Therefore, the aim of this study was to examine the effects of SH or high fat KM in a LFM diet for red seabream compared to a LFM and HFM diets and, meanwhile, compare the supplemental effects of SH with KM in LFM diets for red seabream.

2. Materials and Methods

Experimental diets

Four experimental diets were formulated to be isonitrogenous (45% crude protein) and isocaloric (18 kJ g-1) (Table 1) by inclusion of shrimp protein hydrolysates and high fat krill meal those were provided by DIANA AQUA (Aquaculture Division of DIANA, Member of SYMRISE Group, Elven, France). A FM-based diet (40%) was regarded as a high FM diet (HFM) and a diet contained SPC, as a substitute for FM, at replacement level of 37.5% was considered as low-FM diet (LFM). Two other experimental diets (SH and KM) were prepared by dietary inclusion of shrimp protein hydrolysates and high fat krill meal in low FM based diet at 5% inclusion levels in exchange of 12.5% FM from LFM diet to contain 50% of FM from HFM diet.

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Table 4. 1 Formulation and proximate composition of the four experimental diets for red seabream (%, dry matter basis)

	Experimental diets			
	HFM	LFM	KM	SH
Morocco fish meal	40.0	25.0	20.0	20.2
Krill meal	0.0	0.0	5.0	0.0
Shrimp hydrolysate	0.0	0.0	0.0	5.0
Soy protein concentrate (SPC)	9.0	21.0	21.0	21.0
Corn gluten meal	8.0	8.0	8.0	8.0
Wheat flour	30.5	29.8	29.8	29.6
Squid liver oil	4.0	5.5	5.2	5.5
Soybean oil	4.0	4.0	4.0	4.0
Mineral Mix[1]	1.0	1.0	1.0	1.0
Vitamin Mix[2]	1.0	1.0	1.0	1.0
Starch	2.0	1.5	1.8	1.5
Choline chloride	0.5	0.5	0.5	0.5
Lysine	0.0	0.5	0.5	0.5
Methionine	0.0	0.2	0.2	0.2
Taurine	0.0	0.5	0.5	0.5
Di-calcium phosphate	0.0	1.5	1.5	1.5
Proximate composition				
Dry matter	91.1	91.2	91.0	90.6
Crude protein	46.9	47.1	48.2	47.9
Crude lipid	14.6	14.4	14.6	14.4
Ash	9.0	8.1	7.7	8.0

[1]Mineral premix (g kg^{-1} of mixture): $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3$. $6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[2] Vitamin premix (g kg^{-1} of mixture): L-ascorbic acid, 121.2; DL-α tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-D-pantothenate, 12.7; myo-inositol, 181.8; D-biotin, 0.27; folic acid, 0.68; p-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

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Table 4. 2. The molecular weight distribution of shrimp hydrolysate (SH) (%).

Molecular weight (Da)	SH
>30,000	<0.1
20,000–30,000	<0.1
10,000–20,000	<0.1
5000–10,000	<0.5
1000–5000	8
500–1000	9
<500	83

Table 4. 3. Proximate composition of shrimp hydrolysate (SH) and krill meal (KM) (from product technical data sheets).

	SH	KM
Dry matter, DM (%)	96.7	92.6
Protein (% DM)	67.2	65.0
Lipid (% DM)	12.2	24.0
Ash (% DM)	10.3	11.8
Soluble protein (% total protein)	94.0	13.0
Total PUFA[1] (% fatty acids)	40	23
Total omega 3(% fatty acids)	15	20
EPA[2] (% fatty acids)	6	10
DHA[3] (% fatty acids)	6	5
Essential amino acids (% products)		
Arg	4.1	3.8
His	1.5	1.5
Ile	2.7	3.5
Leu	4.3	5.3
Lys	4.1	4.9
Met	1.3	2.1
Phe	2.9	3.1
Thr	2.4	3.0
Val	3.3	3.5

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Feeding trial and sample collection

The feeding trial was conducted in the Marine and environmental research institute, Jeju National University, Jeju, Korea. All fish were acclimatized for two weeks by feeding a commercial diet. Fish (8.5±g) were randomly distributed among twelve polyvinyl circular tanks (30 fish / tank) those were randomly assigned to three replicates of four dietary treatments and supplied with filtered sea water at a flow rate of 3 L min^{-1} and aerated by sandstone to maintain sufficient dissolved oxygen. Fish were fed the experimental diets twice a day (09.00h and 18.00h) until satiation for fifteen weeks. Growth of fish was measured in three weeks interval. Feeding was stopped 24 hour prior to weighting to minimize stress. Uneaten feed was collected 30 min after feeding and reweighed to determine feed intake. Experimental protocols followed the guidelines of the Animal Care and Use Committee of Jeju National University.

Sample collection and Analyses

At the end of the feeding trial, all the fish in each tank were counted and bulk weighted for calculation of growth parameters. Three fish per tank (nine fish per dietary treatment) were collected for whole-body proximate composition analysis. Also, three fish per tank were randomly captured, anesthetized with 2-phenoxyethanol solution (200 ppm) and blood samples were taken from the caudal vein with heparinized syringes. After analysis of hematocrit, hemoglobin and respiratory burst activity in whole blood samples, plasma was separated to be used for determination of biochemical parameters. Another set of blood samples were taken with non-heparinized syringes and allowed to clot at room temperature. Then centrifuged for 10 min at 5000×g to separate serum and stored at −80 °C for the analysis of innate immune parameters. Feeding was stopped 24 h prior to weighing or blood sampling to minimize the stress of fish.

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Analysis of moisture and ash content were performed by the standard procedures (AOAC, 1995). Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FOSS, Sweden) and crude lipid was determined according to Folch et al. (1957). Hematocrit was determined by microhematocrit technique (Brown, 1980). Hemoglobin and plasma biochemical parameters (glucose, total protein, cholesterol, and triglyceride) were determined by an automated blood analyzer (SLIM, SEAC Inc, Florence, Italy).

The oxidative radical production by phagocytes during respiratory burst was measured through NBT assay described by Anderson and Siwicki (1995). Serum myeloperoxidase (MPO) activity was measured according to Quade and Roth (1997). Serum superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase using a SOD Assay Kit (Sigma, 19160) according to the manufacturer's instructions. Catalase activity was assayed using a kit (K773, Biovision, Inc. California, USA). GPx activity was assayed with a kit (K762, Biovision, Inc. California, USA) using cumene hydroperoxide as a peroxide substrate.

Estimation of apparent digestibility coefficients

Chromic oxide (Cr_2O_3) (Sigma-Aldrich, St. Louis, USA) was included in the diets as an inert indicator at a concentration of 1.0% for estimation of apparent digestibility coefficient of the experimental diets according to the method described in our previously published paper (Khosravi et al., 2015). New sets of red seabreams (50g) were used for the digestibility test in a fecal collection system (Guelph system) tanks at a density of 50 fish per tank.

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Expression levels of liver IGF-I mRNA

Liver samples were taken from three fish per tank and frozen immediately in liquid nitrogen. Total RNA isolation and gene expression determination were conducted according to Kim et al.(2017). The 18S rRNA gene was used as the housekeeping gene. Primers were designed using the cloned sequence for both IGF-1 and 18S rRNA gene (NCBI Genbank accession no: AY996779 and AB259837). The relative expression ratio was calculated according to the mathematical model explained by Pfaffl (Pfaffl, 2001): Ratio = [(EIGF-I) ΔGF (control-sample)]/ [(Eactin) Ct (control-sample)].

Statistical analysis

All experimental diets were assigned using a completely randomized design. Data were analyzed by one-way analysis of variance (ANOVA) in SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) to identify differences among groups. The differences in mean values were compared using Tukey's HSD test at the 5% level of significance ($P < 0.05$). Data are presented as mean ± SD.

3. Results

All diets were well accepted and consumed by the fish during the feeding trial. FBW, WG, SGR, FCR and PER were significantly improved in fish fed diets containing HFM, KM or SH compared to those of fish fed LFM diet. Interestingly, the parameters were significantly higher in fish fed SH diet than fish fed KM and HFM diets. Also, FI of fish fed SH diet was significantly higher compared to that of the LFM group. Survival of fish was not significantly affected by the dietary treatments (Table 4).

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DM and protein digestibility of diets were significantly improved by inclusion of SH and KM. (Table 5). Protein digestibility of SH diet was significantly higher than KM, HFM and LFM diets. Protein digestibility of KM and HFM diets were significantly higher than LFM diet. DM digestibility of SH diet was comparable to HFM diet and significantly higher than KM and LFM diets. Also, DM digestibility of HFM and KM diets were similar. The lowest protein and DM digestibility were shown in LFM diet.

The results of innate immune analyses are shown in Table 6. Innate immune responses of fish fed SH diet were significantly enhanced compared to that of the LFM diet. Even though SH group showed the most improved innate immunity, NBT and SOD activities of HFM and KM groups were significantly elevated than the LFM group. However, catalase and GPx activities of KM group were not significant compared to LFM group. No significant differences were found in MPO activity among all the groups.

The results of hematological and biochemical parameters are shown in Table 7. Hemoglobin level of fish fed SH and KM diets were significantly higher than that of fish fed LFM diet. However, hematocrit, glucose, total protein and cholesterol levels of fish were not significantly affected by the diets. No significant differences were observed in whole-body composition of fish (Table 8). However, liver IGF-1 mRNA expression of fish fed SH diet was in line with HFM group and significantly higher compared to that of fish fed KM and LFM diets. KM group showed significantly higher expression level compared to LFM group. The lowest expression level was observed in fish fed LFM diet (Figure 1).

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Table 4. 4. Growth performance and feed utilization of red seabream fed the four experimental diets for 15 weeks

	HFM	LFM	KM	SH
IBW[1] (g)	8.48±0.06	8.53±0.06	8.45±0.07	8.38±0.04
FBW[2] (g)	65±2.55[b]	52.9±1.68[c]	63.2±3.51[b]	76.3±4.79[a]
WG[3] (%)	666±29.4[ab]	520±15.6[c]	649±47.9[ab]	810±60.7[a]
FI[4]	91.3±3.94	93.1±0.91	97.6±5.48	95.1±6.46
FCR[5]	1.60±0.08[bc]	2.01±0.05[a]	1.74±0.14[b]	1.51±0.05[a]
PER[6]	1.33±0.07[ab]	1.06±0.03[c]	1.19±0.09[b]	1.39±0.05[a]
SGR (%)[7]	1.94±0.04[b]	1.74±0.02[c]	1.92±0.06[b]	2.10±0.06[a]
Survival (%)	90.0±5.77	84.4±1.92	84.4±5.09	90.0±3.33

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Initial body weight

[2]Final body weight

[3]Weight gain = [(final body weight - initial body weight)/ initial body weight] x 100

[4]Feed intake (g/fish) = dry feed consumed (g)/fish

[5]Feed conversion ratio = dry feed fed/wet weight gain

[6]Protein efficiency ratio = fish weight gain (g)/protein

[7]Specific Growth Rate = ln (final weight in grams) - ln (initial weight in grams) x100

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Table 4.5. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for red seabream.

	HFM	LFM	KM	SH
ADCd[1] (%)	67.3 ± 1.2^{ab}	56.7 ± 2.8^{c}	64.7 ± 2.4^{b}	70.0 ± 0.9^{a}
ADCp[2] (%)	91.7 ± 0.4^{b}	86.8 ± 1.5^{c}	91.0 ± 0.2^{b}	93.6 ± 0.4^{a}

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$).

[1] Apparent digestibility coefficient of dry matter

[2] Apparent digestibility coefficient of protein

Table 4. 6. Hematological parameters of red seabream fed the four experimental diets for 15 weeks.

	HFM	LFM	KM	SH
Hematocrit (%)	37.6±4ab	32.7±0.9b	37.6±2.8a	35.8±1.3a
Hemoglobin (g dL^{-1})	5.89 ± 0.2^{ab}	4.86 ± 1.06^{b}	6.96±0.77a	6.58 ± 0.27^{a}
Glucose (mg dL^{-1})	57.6±1.0	55.5±1.6	55.8±3.8	57.6±3.0
Total protein (g dL^{-1})	1.87±0.15	1.43±0.04	1.68±0.47	1.94±0.09
Total cholesterol (mg dL-1)	220±12	210±6	217±13	231±13

Values are mean of triplicate groups and presented as mean ± S.D. Values with different superscripts in the same row are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

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Table 4. 7. Non-specific immune response of red seabream fed the four experimental diets for 15 weeks.

	HFM	LFM	KM	SH
NBT[1]	0.95±0.07[ab]	0.71±0.04[c]	0.84±0.07[b]	1.02±0.06[a]
MPO[2]	1.66±0.13	1.36±0.11	1.76±0.44	1.81±0.09
SOD[3]	74.8±0.3a[b]	63.3±2.7[c]	74.6±2.2[b]	81.6±4.1[a]
GPx[4]	109±17[a]	75.9±7.9[b]	95.5±20[ab]	116±8.8[a]
Catalase[5]	1.35±0.09[ab]	1.05±0.09[c]	1.19±0.07[bc]	1.41±0.14[a]

Values are mean of triplicate groups and presented as mean ± SD. Values in the same row having different superscript letters are significantly different ($P < 0.05$). The lack of superscript letter indicates no significant differences among treatments.

[1]Nitro blue tetrazolium activity

[2]Myeloperoxidase level

[3]Superoxide dismutase (% inhibition)

[4]Glutathione peroxidase activity (mU ml^{-1})

[5]Catalase activity (mU ml^{-1})

Table 4. 8. Whole-body composition of red seabream fed the four experimental diets for 15 weeks (%, DM).

	HFM	LFM	KM	SH
Dry matter	30.0±0.41	32.7±1.20	32.9±0.60	33.1±1.59
Protein	53.6±0.4	51.8±0.3	53.6±1.4	54.0±0.2
Lipid	34.1±2.5	32.8±2.5	33.4±2.1	33.8±0.8
Ash	13.9±1.0	11.4±0.6	13.7±1.4	13.8±0.8

Values are mean of triplicate groups and presented as mean ± S.D.

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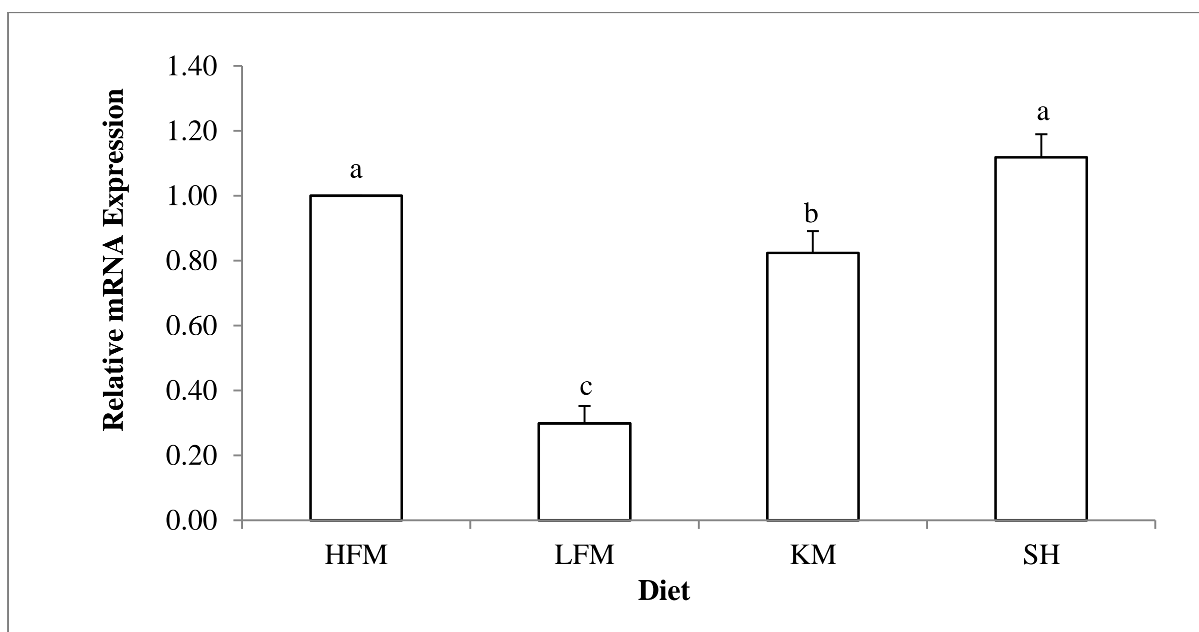

Figure 4. 1. Liver insulin-like growth factors I mRNA expression for each diet group expressed as a ratio to HFM diet values. Data are presented as mean ± SD from three replicate tanks. Different letters above the bars denote significant differences between diet groups at the $P < 0.05$ level.

4. Discussion

Dietary SH supplementation in a LFM diet was able to restore fish growth and exerted even better performance than the HFM group. Also, HFM effects were similarly restored in fish by KM inclusion into LFM diet. In our previous studies, growth performance of red seabream was improved by supplementation of 5% SH compared to a HFM diet (Bui et al., 2014). HFM effects were also restored in red seabream by SH supplementation replacing 50% FM with SPC (Khosravi et al., 2015). However, FI was not significantly affected by the supplementations. Therefore, increased growth performance by SH or KM supplementation might be due to the increased palatability and/or nutritional quality of diets which was represented by improved FCR and PER compared to the LFM group. KM and SH

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have well balanced essential amino acids (AA) and fatty acids for fishes. Also, soluble protein level is high in SH containing high level of low molecular weight peptides (Table 2, 3). Many active peptides, known as growth promoter in fish, are found in these molecular ranges (Chalamaiah et al., 2012). Also, crude lipid level and omega-3 fatty acids, especially EPA and DHA, are high in KM. SH and KM contain astaxanthin which is a growth stimulator of fish (Kalinowski et al., 2011; ; Li et al., 2014; Xie et al., 2017; Cheng et al., 2018; Huang et al., 2018; Lim et al., 2018). Therefore, these compounds might be involved in restoring HFM effects by SH or KM supplementation into the LFM diet. However, low molecular compounds and peptides which are generated during the process of hydrolysis might be a reason for the improved growth performance of SH fed fish compared to KM fed fish. Teshima et al. (2004) reported that dietary supplementation of low molecular weight peptides enhances the growth performance of red seabreams. Kondo et al. (2017) demonstrated that feed conversion and expression of digestive enzymes of red seabream can be improved by dietary protein hydrolysate supplementation.

In our previous studies, SH supplementation improved protein and DM digestibility of red seabream compared to a HFM diet (Bui et al., 2014). Digestibility of red seabream diet is dependent on several factors such as feeding rate (Takii ettt al., 1997), photoperiod (Biswas et al., 2005) and stocking density (Biswas et al., 2007). Dossou et al. (2018) reported that FM can be replaced up to 25% with fermented rapeseed meal without compromising digestibility of protein in red seabream diet. Kader and Koshio. (2012) improved diet digestibility by incorporating 20% of FM with fermented soybean meal and fish soluble in red seabream diet. Protein and AA digestibility of red seabream vary with protein sources in diet (Yamamoto et al., 1998). Therefore, in line with these evidences, it can be assumed that proper level of SH

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in a LFM diet for red seabream possess an ability to enhance protein digestibility of the diet. KM supplementation was reported increased digestibility of no-FM diet for Nile tilapia (*Oreochromis niloticus*) (Gaber et al., 2005). Tibbetts et al. (2006) observed that protein digestibility of Atlantic cod (*Gadus morhua*) was improved by KM supplementation. However, in our study, protein digestibility was higher in SH diet than that of KM diet. Nutritional quality of SH, especially higher levels of low molecular weight peptides, seems to the main reason for the improved protein digestibility of diet compared to KM or HFM diets.

Innate immune system is the fundamental defense mechanism found in fish for combating infections. It is divided into three main components as physical parameters, cellular or humoral factors (Magnadóttir, 2006). Humoral factors are included with cell associated receptors and soluble molecules in body fluids. In the present study, humoral parameters such as serum catalase, SOD and GPx activities were significantly increased by dietary SH and KM supplementation in a LFM diet. SH supplementation resulted in even higher performance than HFM group. According to our previous studies, innate immune parameters of red seabream were improved by SH supplementation into a LFM diet restoring the positive HFM effects (Khosravi et al., 2015Innate immune system of olive flounder (*Paralichthys olivaceus*) was also stimulated by dietary SH supplementation (Khoshravi et al., 2017). Innate immunity of seabass and Atlantic halibut was enhanced by dietary supplementation of hydrolysates from Pollock by-product (Liang et al., 2006; Hermannsdottir et al., 2009). Siddik et al. (2019) reported that immune responses of Asian seabass (*Lates calcarifer*) can be improved by tuna hydrolysates supplementation in diets. Immunostimulatory effects of different hydrolysates products have been revealed over past decade (Huang et al., 2014; Chalamaiah et al., 2015; Karnjanapratum et al., 2016;

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Chalamaiah et al., 2018). In this regards, immune parameters of red seabream might be more improved by dietary SH supplementation compared to KM which was not hydrolyzed. Limited information is available on the mechanism of action for protein hydrolysates to stimulate and improve fish immunity. Evidences from previous studies elucidated that chitin present in KM have ability to improve immunity of fish (Ringo et al., 2012).

IGF-1 is a mitogenic polypeptide that can mediate somatic growth-promoting effects of growth hormone. It is secreted by the liver and transported to other tissues in vertebrates (Mauras, 1997; Laron, 2001). In the same way, IGF-1 in teleost is regulated by growth hormone and secretion of hepatic IGF-1 in a dose-dependent manner (Shamblott et al., 1995). IGF-1 plays an important role to develop nervous system and muscle during the larval stage of Chilean flounder (*Paralichthys adspersus*), (Escobar, 2011). Furthermore, McCormick, (1996) demonstrated that this gene might be involved in osmoregulation of Atlantic salmon. In the present study, relative expression levels of liver IGF-1 mRNA of LFM fish group did not reach to those of HFM fish group. However, KM supplementation resulted in upregulated expression level of the LFM group and dietary SH supplementation restored the HFM effects. Bjørndal et al. (2012) observed that dietary krill powder can upregulate the expression of animal genes. Zheng et al. (2012) found that liver IGF-1 expression of Japanese flounder was significantly increased by replacing 16% FM with protein hydrolysates. The present results along with the previous ones indicate that the SH, as a hydrolysate, could recover a decreased IGF-1 gene expression of fish fed a diet containing high plant protein sources even though the exact mechanism has not been clearly verified. Plasma IGF-1 level was also found to have a correlation with the protein contents of isocaloric diets (Moriyamaet al., 2000). SH and KM used for this study contain almost similar level of amino acids proteins. Whereas,

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soluble protein level was higher in SH compared to KM. therefore high levels of soluble protein in SH might explain the reason for the increased IGF-I expression.

In the present study hematocrit and hemoglobin level of fish significantly improved by dietary KM and SH supplementation compared to LFM group. Olsen et al. (2006) reported that dietary KM supplementation had no adverse effects on hematocrit, hemoglobin or red blood cell count of Atlantic salmon. Also, Silva-Carrillo et al. (2012) replaced FM from juvenile spotted rose snapper (*Lutjanus guttatus*) diet with soybean meal up to 60% without adverse effects on hematocrit and hemoglobin level when diets were supplemented with KM and Squid meal. Khosravi et al. (2015) observed increment in blood hemoglobin and hematocrit by dietary SH supplementation. High level of plant protein in diets causes to reduce hemoglobin level and hematocrit of fish (Haghbayan et al., 2015). Therefore, in our study, blood hemoglobin level and hematocrit were reduced by LFM diet due to high level of SPC and might be increased by KM and SH to reach HFM effects.

In conclusions, the results observed by this study provide evidences to prove that the 20% of FM inclusion in red seabream diet with KM is comparable to a HFM diet which contain 40% FM. Also, compared to KM, SH can be used to replace FM from red seabream diet down to 20% and fish performance can be maintained better than a 40% FM containing diet. Therefore, inclusion of SH in LFM diet is effective than KM to improve growth, digestibility and immunity of red seabream and further investigations are required to improve fish diets with SH.

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Chapter V

Effects of different dietary protein hydrolysates on growth, feed utilization, intestinal morphology, innate immunity and resistance to *Vibrio harveyi* in Pacific white shrimp

1. Introduction

PWS is native to Eastern Pacific Ocean and, commonly caught or farmed as a food. Commercial production of PWS began in 1970 and its production was exceeded over 50% of total crustacean aquaculture production since 2016 (FAO, 2018). PWS are an omnivorous species. However, inclusion of marine protein sources in their diet is required for a better feed utilization in its commercial aquaculture (Amaya et al., 2007; Suárez et al., 2009; Bauer et al., 2012). Therefore, in commercial feed, marine protein sources such as FM, shrimp meal and squid meal are added around 25% by weight (Tacon and Barg, 1998).

Squid meal or squid liver powder and viscera meal, by product of squid or cuttlefish processes, is an appetizer and a feed attractant used in fish and shrimp diet (Liang et al., 2000; Suresh et al., 2011). Growth and feed utilization of fish and shrimp can be improved by dietary supplementation of ingredient produced by squid processing by-products. Growth of Japanese seabass (*Lateolabrax japonicas*) was improved by dietary squid viscera meal supplementation compared to a high FM diet (Mai et al., 2006). Growth performance and trypsin and chymotrypsin activities of PWS were increased by dietary squid meal supplementation up to 9% and decreased when 15% of total protein was supplemented with squid meal in the diet (Córdova-Murueta et al., 2002). Similarly, dietary squid meal enhanced growth of giant tiger prawn (*Penaeus monodon*) (Smith et al., 2005). Cruz-Ricque et al. 1989

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revealed a positive effect of squid extract on growth, feed utilization and nutrient absorbance of Kuruma prawn (*Penaeus japonicas*). However, high levels of dietary squid product supplementation were reported to suppress shrimp growth (Córdova-Murueta et al., 2002; Smith et al., 2005). Also, it was suggested that long term feeding of squid product may result in cadmium accumulation in animal tissues (Mai et al., 2006).

TuHs are produced from fish processing waste (viscera, head or frame) which are rich source of protein and have well balanced amino acid. In the case of fish, amount of waste depends on the processing method. Production of canned tuna generates 50 to 70% of raw material as solid waste (Saidi et al., 2014). Therefore, different types of processing waste are available and, used to produce tuna hydrolysates (Guerads et al., 2002; Nguyen et al., 2012; Cheng et al., 2015). Hydrolysates from tuna liver showed antioxidant and antihypertensive properties indicating as a good food ingredient (Je et al., 2009). Tuna frame protein hydrolysates is a beneficial ingredient which contain peptides to act as nutraceuticals and pharmaceuticals against diseases related to hypertension (Lee et al., 2010). Similarly, hydrolysates from bigeye tuna (*Thunnus obesus*) dark muscle were also beneficial against hypertension (Qian et al., 2007). Herpandy et al. (2011) reported that tuna hydrolysate exhibits functional properties (whipping, gelling and texturing) as a food ingredient. Therefore, TuH is used as a protein source in fish and shrimp diets due to their excelent properties as well as valuable protein and AA sources. In a previous study, we observed that TuH improves growth, feed utilization, immunity, digestibility and disease resistance of red seabreams (*Pagrus major*) (Khosravi et al., 2015). In shrimp, growth performance was improved by dietary tuna head hydrolysate supplementation (Nguyen et al., 2012). Hernández et al. (2011) identified that tuna by-product hydrolysates provide palatability to shrimp feed

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leading to while improved protein digestibility and amino acid profile of feed. Also, they observed that the growth performance of fish was positively influenced after six weeks of a feeding trial.

Fish soluble is a by-product generated during fish manufacturing processes such as canning, FM and fish oil production. The soluble fraction is centrifuged to remove oil fraction and then use a fraction containing water to produce condensed or DFS after drying. As a feed ingredient in aquaculture, it is reported that fish soluble supplementation to red drum (*Sciuenops ocellatus*) diets was beneficial to improve growth and feed utilization (McGoogan and Gatlin, 1997). Also, fish soluble is recommended to be used in high plant protein diets for Kuruma shrimp to replace FM and improve growth performance. Shrimp hydrolysates are produced by shrimp processing waste which account for 35-45% of whole body weight as inedible waste (Meyers 1986; Shahidi & Synowiecki, 1991). In early studies, Ingredients prepared by shrimp by products (shrimp meal and shrimp head meal) have been used and assayed as a protein source in shrimp diet (Lim et al., 1990; Brunson et al., 1997).

Therefore, in this study, hydrolysate products were included in a high soybean meal diet for PWS and supplemental effects were examined on growth, immunity, digestibility and disease resistance comparing to a SLP supplemented diet.

2. Materials and Methods

Experimental diets

Six experimental diets were formulated to be isonitrogenous (33.3% crude protein) and isocaloric (17.4 kJ g^{-1}). A diet containing 4.49% of squid liver powder was regarded as

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the control diet and three other diets were prepared by supplementing tuna hydrolysate, shrimp hydrolysate and dryied fish soluble provided by DIANA AQUA (Aquaculture Division of DIANA, Member of SYMRISE Group, Elven, France) (designated as SLP, TunaH, SH and DFS respectively). All dry ingredients were thoroughly mixed with fish oil, soybean oil and double distilled water by a mixer machine (NVM-14, Daeyung Co., South Korea) (Fig. 1). Then, the mixed dough was pelleted through a pellet machine (SP-50, Gumgang ENG, Daegu, Korea). The pellets were subsequently dried in 25 ℃ for 8 hours and then stored at –20 ℃ until use. Dietary formulation, proximate and amino acid composition of the experimental diets were provided in Table 1, and 2, respectively.

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Table 5. 1. Formulation of the four experimental diets for Pacific white shrimp (%, dry matter basis).

Ingredients	SLP	TuH	SH	DFS
Tuna meal (55% CP)	9.10	9.10	9.10	9.10
Squid liver powder	4.49			
Krill meal				
Tuna hydrolysate		1.14		
Shrimp hydrolysate			0.94	
Dried fish soluble				1.94
Krill hydrolysate				
Wheat gluten	4.50	4.50	4.50	4.50
Soybean meal	45.10	48.60	48.30	46.90
Wheat flour	16.00	16.00	16.00	16.00
Starch	7.18	6.48	6.94	7.48
Soybean oil	2.00	2.00	2.00	2.00
Fish oil	2.38	3.06	3.11	2.96
Lecithin	1.00	1.00	1.00	1.00
Mineral mix[1]	2.00	2.00	2.00	2.00
Vitamin mix[2]	1.00	1.00	1.00	1.00
Cholesterol	0.04	0.08	0.07	0.08
Choline chloride	1.00	1.00	1.00	1.00
Monocalcium phosphate	3.00	3.00	3.00	3.00
Guar gum	1.00	1.00	1.00	1.00
⋆ Proximate composition result (% DM)				
Crude protein	32.90	33.60	33.80	32.50
Crude lipid	8.00	7.90	8.00	7.20
Ash	6.20	6.40	6.70	6.40
Dry matter	92.80	93.20	93.20	92.80
P (Phosphorus, %)	1.86	1.84	1.83	1.84
Ca (Calcium, %)	1.90	1.94	1.96	1.96
Cholesterol (%)	0.15	0.15	0.15	0.15

[1] $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3$. $6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[2] L-ascorbic acid, 121.2; DL-α tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-$_D$-pantothenate, 12.7; myo-inositol, 181.8; $_D$-biotin, 0.27; folic acid, 0.68; p-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

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Table 5. 2. Amino acid composition (% in sample) of the four experimental diets for Pacific white shrimp.

	SLP	TuH	SH	DFS
Aspartic acid	3.68	3.6	3.34	3.55
Threonine	1.33	1.31	1.23	1.3
Serine	1.69	1.64	1.53	1.62
Glutamic acid	7.39	7.28	6.84	7.24
Proline	2.19	2.48	2.50	2.33
Glycine	1.69	1.67	1.58	1.76
Alanine	1.61	1.54	1.46	1.61
Valine	1.73	1.71	1.61	1.72
Isoleucine	1.6	1.59	1.49	1.58
Leucine	2.65	2.65	2.48	2.61
Tyrosine	1.02	1.08	1.00	1.05
Phenylalanine	1.76	1.78	1.66	1.75
Histidine	1.19	1.15	1.07	1.19
Lysine	1.18	1.14	1.08	1.18
Arginine	1.45	1.34	1.27	1.4

Shrimp and feeding trial

Shrimp were transported from hatchery to Marine Sciences Research Institute of, Jeju National University (Jeju, Korea) and fed a commercial diet for two weeks to be acclimated to the experimental conditions and facilities. At the end of the acclimation period, shrimp (average body weight, 0.35±0.00 g) were randomly distributed into 110 L capacity 30 acrylic tanks at a density of 25 shrimp per tank those were supplied with filtered seawater and aeration to maintain enough dissolved oxygen. Water temperature of tanks was maintained at 27-30℃ range during the feeding trial. Five replicate groups of shrimp were fed one of the six experimental diets at a ratio of 6-16% body weight (four times a day, 08:30, 12:00, 16:00 and 19:30 h) for 52 days. The rearing water was exchanged every 3 days. Water quality was maintained within a standard range for *L. vannamei* during the experiment period

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Sample collection

At the end of the feeding trial, all the shrimp in each tank were bulk-weighed and individual-weighed for calculation of growth parameters and survival (Fig. 3). Four shrimp per tank (thirty shrimp per dietary treatment) were randomly captured and placed in ice water for 5 min to anesthetize before hemolymph sampling. Hemolymph was withdrawn from the ventral sinus of each shrimp into a 1 mL syringe (25gauge needle) containing 400 µL of precooled (4°C) anticoagulant solution (Alsever's solution, sigma) (Fig. 4). The diluted hemolymph from each shrimp was kept and analyzed separately. About 500 µl anticoagulant-hemolymph was used to determine nitroblue-tetrazolium (NBT), total haemocyte count (THC) and hyaline cell count. The remaining anticoagulant-hemolymph mixture was centrifuged at 800 × g for 20 min at 4℃ and the supernatant was stored at -70℃ for immune related analyses. Feeding was stopped 24 h prior to weighing or blood sampling to minimize stress.

Estimation of apparent digestibility coefficients

For estimation of apparent digestibility coefficient of the experimental diets, chromic oxide (Cr_2O_3) (Sigma) were included in the diets as an inert indicator at a concentration of 10 g kg^{-1} diet. Another set of shrimp were stocked into the 240 L capacity tanks at a density of 40-50 shrimp per tank. Fecal samples were collected three times a day from each tank (11:30, 15:00 and 18:30 hours). All feces were collected from each tank which were pooled and later frozen at -40 ℃ until analyses. Chromium oxide content of diet and feces samples were analyzed by the method described by Divakaran et al. (2002).

Analyses

<u>Water quality parameters</u>

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During the 10 week growing period, the water quality parameters such as dissolved oxygen (Orion Star A216 Benchtop, Thermo Scientific) and pH (Seven Compact, METTLER TOLEDO) were monitored. NH_4^+ was analyzed by the method described by Verdouw et al. (1978).

Proximate composition analysis

Analysis of moisture, ash content and the muscle water retention rate were performed by the standard procedures (AOAC, 2005). Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FOSS, Sweden) and crude lipid was analyzed by the method described by Folch et al. (1957).

Immunological assays

The anti-protease activity was measured according to the method described by Ellis (1990), with slight modifications (Magnadóttir et al., 1999). Hemolymph superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase using a SOD Assay Kit (Sigma, 19160) according to the manufacturer's instructions. GPx activity was assayed using kit (Biovision, Inc. California, USA). A turbidometric assay was used for determination of serum lysozyme level through the method described by Hultmark (1980) with slight modifications. Catalase activity was assayed using kit (Biovision, Inc. California, USA). Total antioxidant capacity (TAC) was determined using a commercial kit (Antioxidant assay kit, Sigma; CS0790).

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Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA, Sigma) using the method described by Hernández-López et al (1996). In brief, 50μL of sample was placed in a 96-well plate and pre-incubated with 50 μL of trypsin (1mg mL-1 in CAC buffer) (Sigma) for 30 min at 25℃. Then, 50 μL of L-DOPA (3mg mL-1 in CAC buffer) was added. The CAC buffer includes 10mM sodium cacodylate and 10mM $CaCl_2$, pH 7.0. After 10 min of incubation at 25℃, the absorbance was recorded at 490 nm using a microplate reader (UVM 340, Biochrom, Cambridge, UK). The optical density of the shrimp's phenoloxidase activity was expressed as dopachrome formation in 50 μl of cell-free hemolymph.

Oxidative radical production by phagocytes during respiratory burst was measured through NBT assay described by Zhang et al (2013). HBSS solution of 200ml was added into a separate 2ml Eppendorf tube (for triplicate). Then, 40 ml of hemolymph diluted in anticoagulant solution (4:1) was transferred in Eppendorf tube and incubated for 30 min at 25℃. Next, 100 ml zymosan (0.1% in Hank's solution) was added and incubated for 2 h at 37℃. NBT solution (0.3%) was added and incubated for 2 h at 37℃. Discarded supernatant after 600 ml methanol was added and centrifuged at 6500 rpm for 10 min. After fixed hemocytes was washed three times with 100 ml methanol (70%) and dried for 5 min. Formazan was dissolved with 700ml 2M KOH and 800ml DMSO and absorbance was read at 620 nm using a spectrophotometer.

Hematological parameters

Levels of cholesterol, triglyceride, glucose and total protein were determined by an automated blood analyzer (SLIM, SEAC Inc, Florence, Italy). The total numbers of haemocytes and hyaline cells within the measured hemolymph were measured with a

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haemocytometer using an inverted phase-contrast microscope (Leica DMIL, Leica Microsystems GmbH, Wetzlar, Germany).

Intestine and hepatopancreas morphology

Intestine and hepatopancreas samples were collected from two shrimp per tank and fixed in Bouin's solution. The fixed tissues were dehydrated in ascending concentrations of alcohol, cleared in toluene, embedded in paraffin and sectioned with a rotary microtome at 5μm. Slides were stained with Harris hematoxylin, 0.5% eosin, alcian blue (AB) at pH 2.5 and periodic acid Schiff (PAS) for observation of the villus height and hepatopancreas tubules. Then, the slides were observed to identify hepatopancreas cells(Four cell types dominate the hepatopancreas tubules; namely E "embryonalzellen" or embryonic" cells, R "restzellen" cells, F "fibrillenzellen" or "fibrous" cells, and B "blasenzellen" cells) and measure villus height using the ImageJ 1.44 analysis software (Fig. 7).

Challenge test

At the end of the feeding trial, shrimp from each tank (45 shrimp per treatment) were randomly selected and subjected to a bacterial challenge. *V. harveyi* was used as the pathogenic agent (provided by the Marine Microbiology Laboratory of Jeju National University). The shrimp was injected intramuscularly with *V. harveyi* suspension containing 2×10^5 CFU mL^{-1}. The pathogenic dose of bacterium had previously been determined in a preliminary test using shrimp of a similar size. After injection, the shrimp was distributed into 110 L acryl tanks, and their mortality was monitored and recorded for 19 days.

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Statistical analysis

All experimental diets were assigned using a completely randomized design. Data were analyzed by one-way analysis of variance (ANOVA) in SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). When ANOVA identified differences among groups, the differences in mean values were compared using Tukey's HSD test at the 5% level of significance (P <0.05). Data are presented as mean ± SD. Percentage data were arcsine transformed before analysis.

3. Results

All the experimental diets were readily accepted by the PWS at the start of the feeding trial and they fed aggressively during the 52 days of the feeding trial. Growth performance, feed utilization and survival rate of shrimp fed four experimental diets are shown in Table 5.3. FBW of Shrimp fed the SH diet were significantly higher to that of shrimp fed SLP, TuH and DFS diets. WG of shrimp fed SH diet was significantly higher compared to the other dietary groups. FCR was significantly higher in TuH and DFS groups compared to shrimp in SH group. Therefore, the feed conversion was more efficient in SH group. PER of shrimp fed SH diet was significantly higher compared to TuH group. Survival rates were not significantly influenced by the dietary treatments.

The results of non-specific immune response parameters are shown in Table 5.4. Shrimp fed TuH, SH and DFS diets exhibited significantly higher lysozyme activity than the shrimp fed SLP diet. GPx activity of SLP group was significantly higher than the shrimp in DFS group. TAC was significantly higher in shrimp fed the TuH, SH and DFS diets than the SLP group whiles the highest values were shown by the shrimp fed TuH and SH diets. Catalase activity of shrimp was significantly elevated in the shrimp fed TuH, SH and DFS diets

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compared to SLP group. No significant differences were observed in NBT, SOD, PO and antiprotease activities among all the groups.

The results of hematological parameters are shown in Table 5.5. Glucose level of shrimp fed SLP diet was significantly lower than TuH, SH and DFS groups. Triglyceride level of shrimp in SLP group was significantly lower to SH and DFS groups. No significant differences were observed in, total hemocyte count (THC), hyaline cell count (HC), total protein level and total cholesterol level among all the groups.

The results of whole body proximate and amino acid composition are shown in Table 5.6 and Table 5.7. Shrimp fed TuH, SH and DFS diets exhibited significantly higher lipid composition than shrimp fed SLP diet.

Intestinal and hepatopancreas morphology of shrimp are shown in Table 5.8. No significant differences were observed in hepatopancreas histology. However, villus height of shrimp fed SH diet was significantly higher than shrimp fed SLP, TuH and DFS diets.

The results of apparent digestibility coefficients for dry matter (ADCd), protein (ADCp), lipid (ADCl) and amino acid (ADCa) are shown in Table 5.9 and 5.10. In the case of ADCd, SH diet showed significantly higher ADCd than TuH, and DFS diets. ADCp was significantly higher in SH diet compared to the other diets. ADCl was significantly higher in SLP, TuH and SH diet compared to DFS diet.

At the end of the challenge test, first mortality of shrimp injected with *V. harveyi* was observed after 2 days. However, disease resistance against *V. harveyi* was not significantly affected by any dietary treatments (Figure 5.1).

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Table 5. 3. Growth performance and feed utilization of Pacific white shrimp fed the four experimental diets for 52 days.

	IBW[1]	FBW (g)[2]	WG (%)[3]	FCR[4]	PER[5]	Survival (%)
SLP	0.35±0.00	4.92±0.17[b]	1301±61[b]	1.84±0.12[ab]	1.53±0.09[ab]	79.2±5.93
TuH	0.35±0.00	5.18±0.22[b]	1381±59[b]	1.90±0.10[a]	1.47±0.06[b]	86.4±2.19
SH	0.35±0.00	5.54±0.12[a]	1476±27[a]	1.74±0.05[b]	1.70±0.05[a]	85.6±5.37
DFS	0.35±0.00	5.09±0.18[b]	1338±61[b]	1.90±0.05[a]	1.51±0.04[ab]	83.2±5.22

Values are mean of five replicates groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different (p<*0.05*).

[1]IBW: initial body weight (g)

[2]FBW: final mean body weight (g)

[3]Weight gain (%) = 100 x (FBW – initial mean body weight)/initial mean body weight

[4]Feed conversion ratio = dry feed fed (g) / wet weight gain (g)

[5]Protein efficiency ratio = wet weight gain /total protein given

Table 5. 4. Non-specific immune parameters of Pacific white shrimp fed the four experimental diets for 52 days.

	SLP	TuH	SH	DFS
NBT	2.36±0.82	1.95±0.85	2.38±1.00	2.52±0.73
Lysozyme	4.56±1.19[b]	6.32±1.96[a]	7.03±1.13[a]	7.26±2.49[a]
SOD	85.4±8.4	87.0±4.6	79.2±11.4	81.3±8.5
PO	0.125±0.03	0.125±0.01	0.116±0.02	0.119±0.03
Antiprotease	36.5±2.4	40.7±4.0	39.6±1.9	37.3±2.8
GPx	32.7±6.8[a]	28.7±7.4[ab]	28.1±2.9[ab]	26.3±7.0[b]
TAC	1.04±0.05[c]	1.24±0.05[a]	1.26±0.10[a]	1.14±0.04[b]
Catalase activity	0.96±0.01[c]	1.20±0.04[b]	1.35±0.05[a]	1.43±0.04[a]

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same raw are significantly different ($p < 0.05$).

[1]Nitro blue tetrazolium; phagocytic activity (absorbance)

[2]Lysozyme activity (μg ml^{-1})

[3]Superoxide dismutase (% inhibition)

[4]Phenoloxidase activity (absorbance)

[5]Antiprotease activity (% inhibition)

[6]Glutathione peroxidase activity (mU ml^{-1})

[7]Total antioxidant capacity (μmol ml^{-1})

[8]Catalase activity

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Table 5. 5. Hematological parameters of Pacific white shrimp fed the four experimental diets for 52 days.

	Glucose[1]	Triglycer[2]	Total prot[3]	Total cho[4]	THC[5]	HC[6]
SLP	207±6.2[b]	16.0±2.6[b]	1.74±0.54	7.92±0.70	122±14	66.7±12
TuH	276±29.8[a]	17.6±2.0[ab]	2.15±0.35	7.35±1.51	124±14	68.4±10
SH	300±8.4[a]	21.2±1.3[a]	2.20±0.61	7.45±1.41	128±14	67.8±11
DFS	265±7.5[a]	21.2±2.2[a]	2.35±0.51	8.53±1.53	125±12	69.6±7.0

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

1Glucose (mg dL^{-1})

2Triglyceride (mg dL^{-1})

3Total protein (g dL^{-1})

4Total cholesterol (mg dL^{-1})

[5]Total haemocyte count (10^5 cells ml^{-1})

[6]Hyaline cells count (10^5 cells ml^{-1})

Table 5. 6. Whole body composition (%) without viscera of experimental Pacific white shrimp fed the four experimental diets for 52 days.

	Dry matter	Ash	Protein	Lipid
SLP	24.7±0.04	13.3±0.07	74.2±0.46	3.78±0.16[b]
TuH	24.3±0.49	13.3±0.21	75.5±0.17	5.03±0.03[a]
SH	24.6±0.06	13.0±1.25	76.3±0.81	5.58±0.14[a]
DFS	24.4±0.01	13.6±0.23	74.5±1.49	5.96±0.12[a]

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

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Table 5. 7. Amino acid composition (% in sample) for whole body of experimental Pacific white shrimp fed the experimental diets for 52 days.

	SLP	TuH	SH	DFS
Aspartic acid	8.89	8.83	9.08	9.06
Threonine	3.12	3.02	3.07	3.14
Serine	2.99	2.82	2.88	2.92
Glutamic acid	14.2	14.0	14.4	14.6
Proline	5.14	5.28	4.89	5.42
Glycine	7.74	7.77	8.17	7.85
Alanine	3.34	3.19	3.33	3.44
Valine	3.76	3.77	3.82	3.94
Isoleucine	3.72	3.73	3.82	3.93
Leucine	6.29	6.20	6.37	6.54
Tyrosine	2.59	2.47	2.55	2.65
Phenylalanine	3.47	3.39	3.52	3.63
Histidine	2.38	2.34	2.33	2.45
Lysine	6.92	6.75	6.96	7.12
Arginine	9.43	9.30	9.59	9.43

Table 5. 8. Intestinal morphology of Pacific white shrimp fed four experimental diets for 52 days.

	Villus height, μm
SLP	67.6±5.6[b]
TuH	81.8±11.7[b]
SH	97.0±11.2[a]
DFS	75.0±11.4[b]

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

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Table 5. 9. Apparent digestibility coefficients (%, ADC) for dry matter, protein and lipid of the experimental diets for Pacific white shrimp.

	ADCd (%)[1]	ADCp (%)[2]	ADCl (%)[3]
SLP	83.2±0.2[ab]	90.4±0.1[b]	89.1±0.1[a]
TuH	82.2±0.3[b]	91.1±0.2[b]	89.8±0.2[a]
SH	84.3±2.7[a]	92.4±1.3[a]	89.7±1.8[a]
DFS	81.8±0.4[b]	90.3±0.2[b]	85.5±0.3[d]

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

[1] Apparent digestibility coefficient of dry matter

[2] Apparent digestibility coefficient of protein

[3] Apparent digestibility coefficient of lipid

Table 5. 10. Apparent digestibility coefficients (%, of ADC) for amino acid composition of the four experimental diets for red seabream.

	SLP	TuH	SH	DFS
Aspartic acid	92.5±0.1	93.3±0.3	93.5±0.1	92.3±0.2
Threonine	96.1±0.2	96.5±0.1	96.6±0.0	95.9±0.1
Serine	96.4±0.4	96.7±0.1	96.8±0.1	96.2±0.1
Glutamic acid	90.1±0.4	90.9±0.3	91.2±0.1	89.9±0.4
Proline	96.7±0.2	96.8±0.3	96.4±0.2	96.2±0.2
Glycine	94.8±0.1	95.2±0.2	94.4±0.7	94.3±0.7
Alanine	95.9±1.1	96.4±1.0	96.2±1.0	95.9±0.8
Valine	95.6±0.2	96.0±0.2	96.2±0.2	95.6±0.1
Isoleucine	96.7±0.2	97.0±0.1	97.1±0.2	96.7±0.1
Leucine	94.5±0.3	95.3±0.2	95.4±0.2	94.6±0.2
Tyrosine	97.5±0.2	97.9±0.1	98.0±0.1	97.6±0.1
Phenylalanine	96.3±0.1	96.8±0.1	96.9±0.1	96.3±0.2
Histidine	95.6±1.7	96.2±0.0	96.5±0.6	95.1±0.5
Lysine	96.7±0.1	96.9±0.1	97.1±0.1	96.6±0.2
Arginine	96.4±0.1	96.8±0.1	96.7±0.1	96.3±0.4

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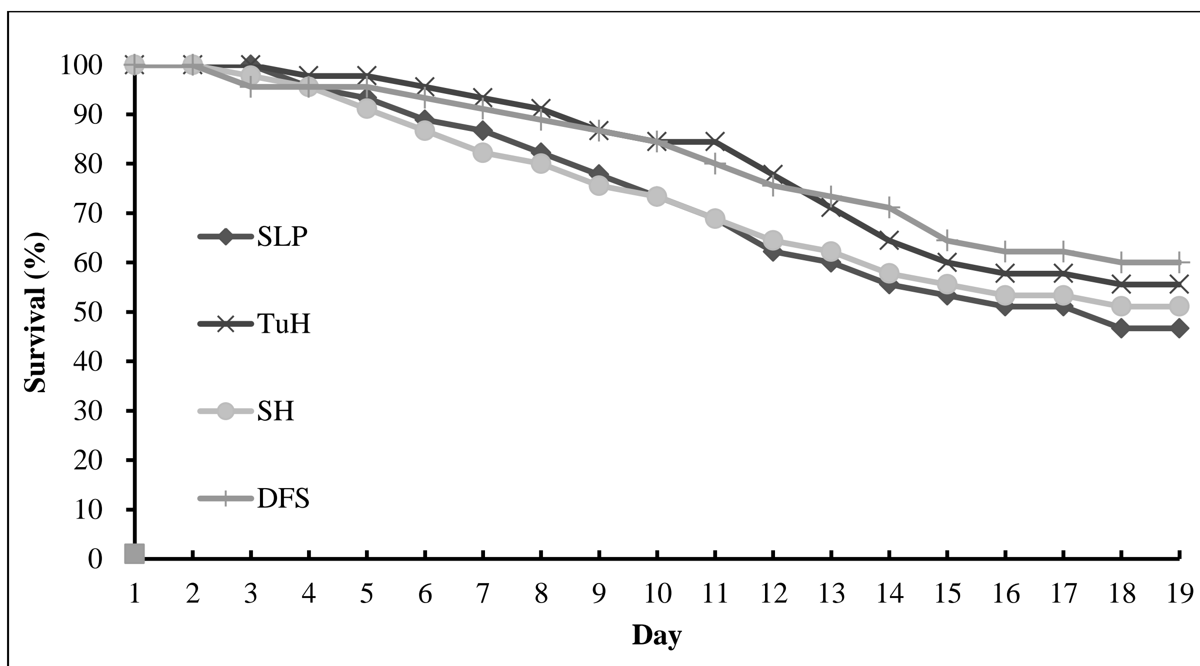

Figure 5. 1. Survival rate of Pacific white shrimp fed the four experimental diets after challenge with *Vibrio harveyi*.

4. Discussion

The results of the present study indicated that the dietary supplementation of SH improves the growth performance and feed utilization of PWS compared to SLP, TuH and DFS. SH has been evaluated in diets for fishes and shrimps, and positive effects were elucidated on growth, immunity, digestibility, palatability and health (Plascencia-Jatomea at el., 2002; Leal at el., 2010; Koshravi et al., 2015a; Koshravi et al., 2015b; Khoshravi et al., 2017; Leduc at el., 2018). SH contains astaxanthin which is a growth stimulator of fish (Kalinowski et al., 2011; Li et al., 2014; Xie et al., 2017; Cheng et al., 2018; Huang et al., 2018; Lim et al., 2018). Dietary supplementation of low molecular weight peptides enhances the growth performance of fish (Teshima et al., 2004). Low molecular compounds and peptides which are generated during the process of hydrolysis might be a reason for the

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improved growth performance of shrimp fed SH compared to SLP group. The growth of shrimp fed TuH diet did not followed this trend although values were numerically higher than SLP and DFS groups. However, in a previous study, we observed that TuH improve growth performance of red seabreams (*Pagrus major*) (Khosravi et al., 2015). Supplementation of TuH is recommended for non-fishmeal based barramundi (*Lates calcarifer*) diets to promote growth performance (Siddik et al., 2019). Therefore, further studies are required to elucidate the effects of TuH on growth performance of PWS. Fish solubles are high in water-soluble vitamins and have been used as a source of unidentified growth factors in poultry and swine feeding (Soares et al., 1970). With water-soluble vitamins, fish solubles are also high in other water-soluble substances with boosted feeding stimuli response, such as amino acids (Smith et al., 2005). However, after the feeding trial, no significant differences were observed in shrimp growth compared to SLP group although numerically higher growth was observed. Further studies are required to evaluate the effects of higher levels of DFS in PWS diets.

The lysozyme GPx and catalase activities were significantly affected by the dietary treatments. Lysozyme is a glycolytic enzyme which is well known for its activity against Gram positive bacteria (Jollès and Jollès, 1984). In Kuruma shrimp lysozyme displayed lytic activities against several Vibrio species and fish pathogens, including *Vibrio penaeicida*, a pathogenic bacteria to the Kuruma shrimp (Hikima et al., 2003). Therefore it is suggested that shrimp lysozyme affects a greater variety of pathogens. GPx is one of the essential components of cellular detoxification systems that defend cells against reactive oxygen species and catalyze the the conjugations of a broad range of electrophilic substances to glutathione (Sheehan et al., 2001, Ketterer, 2001). Catalase is an important antioxidant enzyme and exists virtually in all oxygen-respiring organisms. The main role of catalase is

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eliminating excessive hydroperoxide and maintaining cellular redox balance (Wu and Shah, 1995; Klotz et al., 1997). According to present results, TuH, SH and DFS showed higher lysozyme and catalase activities indicating that the disease resistance can be enhanced by dietary supplementation of these ingredients. Also, TAC was higher in shrimp fed TuH, SH and DFS diets. Therefore, antioxidant status might be improved by these products scavenging reactive oxygen species. The results of challenge test might represent this enhanced immunity of the shrimp.

In conclusion, growth performance and, immunity as well as intestine morphology of PWS were improved by dietary SH supplementation. Therefore, performance of PWS can be effectively improved by dietary shrimp hydrolysates compared to SLP. Further studies are required to evaluate the effects of higher levels of TuH and DFS in PWS diets.

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Chapter VI

Effects of different dietary levels of protein hydrolysates on growth, feed utilization, intestinal morphology, innate immunity and resistance to *Vibrio harveyi* in Pacific white shrimp

1. Introduction

The PWS is the most important crustacean species in aquaculture, accounting for 70% of the total shrimp production in the world. Shrimp culture is highly dependent on FM to provide the quality protein and flavor that attract shrimp to feed and grow. Demand and price of FM is increasing annually (FAO 2018). There is increasing pressure to use alternative protein sources such as animal by-product meals and plant proteins to replace dietary FM (Naylor et al., 2009). Most published research on the use of rendered animal protein ingredients in practical shrimp diets have reported varying degrees of success. However, previous work has shown that an inexpensive and effective way to obtain the desired overall nutrient profile of the finished aquafeed is achieved by the addition of complementary feed ingredient (Hardy, 2006) Quantity and quality of dietary protein are primary factors influencing shrimp growth, nitrogen loading of the culture system and feed costs. Considerable research has been conducted to evaluate the suitability of various feed ingredients as alternative protein sources for FM (Tacon and Akiyama, 1997).

FM is often utilized in aquatic feeds because they are an excellent source of indispensable amino acids, essential fatty acids, vitamins and minerals. Protein ingredients that can be utilized to substitute FMs, either partially or completely include terrestrial plant and animal products, readily available on world markets. Some ingredients, such as protein hydrolysates, have been reported to possess healthy and nutraceutical properties and have

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been used as supplements to enhance food properties (Haard, 2001; Chalamaiah et ei., 2012). Anggawati et al. (1990) used fish hydrolysates for feeds for Penaeus monodon. They found that replacing 3% FM by fish hydrolysate was enough to enhance shrimp growth. Because of their low price and consistent quality, plant proteins are often an economically and nutritionally viable source of protein. However, due to potential problems associated with insufficient levels of indispensable amino acids (e.g., lysine and methionine), anti-nutritional factors and poor palatability, commercial use are often limited.

Supplementation of MPH in fish diets was evaluated in our previous studies and positive effects were observed in terms of growth, feed utilization, innate immunity, intestine morphology, digestibility, IGF- I mRNA expression and disease resistance against pathogens for olive flounder and red seabream (Khosravi et al., 2014; Bui et al., 2014; Khosravi et al., 2015; Khosravi et al., 2017). Supplementary effects of SH, TuH and DFS on growth, immunity, diet digestibility intestine morphology and disease resistance of PWS were examined by the previous study. These ingredients were observed to have positive influence on shrimp. Therefore, this study was conducted to investigate the effects of supplemental level on growth, feed utilization, intestinal morphology, innate immunity and resistance to *Vibrio harveyi* in PWS.

2. Materials and Methods

Experimental diets

Seven experimental diets were formulated to be isonitrogenous (35% crude protein) and isocaloric (17.4 kJ g^{-1}). A diet containing 4.49% of squid liver powder was regarded as

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the control diet and six other diets were prepared by supplementing 2% or 4% dried fish soluble, 1% or 2% shrimp hydrolysate and 1.5% or 3% tuna hydrolysate (designated as SLP, DFS-2%, DFS-4%, SH-1%, SH-2%, TuH-1.5% and TuH-3% respectively). DFS, SH and TuH were provided by DIANA AQUA (Aquaculture Division of DIANA, Member of SYMRISE Group, Elven, France). All dry ingredients were thoroughly mixed with fish oil, soybean oil and double distilled water by a mixer machine (NVM-14, Daeyung Co., South Korea) (Fig. 1). Then, the mixed dough was pelleted through a pellet machine (SP-50, Gumgang ENG, Daegu, Korea). The pellets were subsequently dried in 25 ℃ for 8 hours and then stored at –20 ℃ until use. Dietary formulation, proximate and amino acid composition of the experimental diets were provided in Table 1, and 2, respectively.

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Table 6. 1. Formulation of the seven experimental diets for Pacific white shrimp (%, dry matter basis).

Ingredients	SLP	DFS-2	DFS-4	SH-1	SH-2	TuH-1.5	TuH-3
Tuna meal (55% CP)	9.10	9.10	9.10	9.10	9.10	9.10	9.10
Squid liver powder	4.49						
Dried fish soluble		1.92	3.84				
Shrimp hydrolysate				0.95	1.90		
Tuna hydrolysate						0.41	0.81
Wheat Gluten	4.50	4.50	4.50	4.50	4.50	4.50	4.50
Soybean meal	45.1	46.8	44.1	48.2	46.8	49.1	48.7
Wheat flour	16.0	16.0	16.0	16.0	16.0	16.0	16.0
Starch	7.40	7.58	8.58	7.01	7.54	6.60	6.61
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Fish oil	2.38	3.01	2.8	3.15	3.08	3.2	3.17
Lecithin	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral Mix1	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vitamin Mix2	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.07	0.12	0.11	0.12	0.11	0.12	0.12
Choline chloride	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Guar gum	1.00	1.00	1.00	1.00	1.00	1.00	1.00
⋆ Proximate composition result (% DM)							
Crude protein	38.7	39.0	39.1	38.9	38.7	39.0	39.2
Crude lipid	6.00	6.19	6.09	6.11	6.1	6.13	6.04
Crude ash	10.2	10.3	10.4	10.2	10.3	10.2	10.3
Moisture	8.65	8.62	8.99	8.52	8.81	8.84	8.75

[1] $MgSO_4.7H_2O$, 80.0; $NaH_2PO_4.2H_2O$, 370.0; KCl, 130.0; Ferric citrate, 40.0; $ZnSO_4.7H_2O$, 20.0; Ca-lactate, 356.5; CuCl, 0.2; $AlCl_3$. $6H_2O$, 0.15; $Na_2Se_2O_3$, 0.01; $MnSO_4.H_2O$, 2.0; $CoCl_2.6H_2O$, 1.0.

[2] L-ascorbic acid, 121.2; DL-α tocopheryl acetate, 18.8; thiamin hydrochloride, 2.7; riboflavin, 9.1; pyridoxine hydrochloride, 1.8; niacin, 36.4; Ca-$_D$-pantothenate, 12.7; myo-inositol, 181.8; $_D$-biotin, 0.27; folic acid, 0.68; p-aminobezoic acid, 18.2; menadione, 1.8; retinyl acetate, 0.73; cholecalficerol, 0.003; cyanocobalamin, 0.003.

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Table 6. 2. Amino acid composition (% in sample) of the seven experimental diets for Pacific white shrimp.

	SLP	DFS-2	DFS-4	SH-1	SH-2	TuH-1.5	TuH-3
Aspartic Acid	3.310	3.530	3.470	3.580	3.660	3.600	3.260
Threonine	1.230	1.310	1.280	1.330	1.390	1.330	1.220
Serin	1.530	1.610	1.570	1.650	1.750	1.670	1.590
Glutamic Acid	7.010	7.430	7.310	7.470	7.680	7.510	7.170
Proline	2.070	2.250	2.210	2.200	2.300	2.220	2.270
Glycine	1.540	1.710	1.770	1.650	1.720	1.630	1.640
Alanine	1.490	1.630	1.670	1.620	1.670	1.610	1.590
Valine	1.660	1.760	1.750	1.790	1.800	1.790	1.780
Isoleucine	1.550	1.650	1.620	1.680	1.700	1.680	1.670
Leucine	2.500	2.260	2.620	2.690	2.750	2.710	2.680
Tyrosine	0.860	0.870	0.890	0.900	1.020	1.020	0.970
Phenylalanine	1.650	1.740	1.690	1.760	1.800	1.770	1.750
Histidine	1.100	1.200	1.320	1.210	1.190	1.150	1.190
Lysine	1.910	2.070	2.250	2.080	2.110	2.160	2.000
Arginine	2.100	2.260	2.260	2.360	2.340	2.360	2.340

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Shrimp and feeding trial

Shrimp were transported from hatchery to Marine Sciences Research Institute of, Jeju National University (Jeju, Korea) and fed a commercial diet for two weeks to be acclimated to the experimental conditions and facilities. At the end of the acclimation period, shrimp (average body weight, 0.11±0.00 g) were randomly distributed into 110 L capacity 30 acrylic tanks at a density of 25 shrimp per tank those were supplied with filtered seawater and aeration to maintain enough dissolved oxygen. Water temperature of tanks was maintained at 27-30℃ range during the feeding trial. Five replicate groups of shrimp were fed one of the six experimental diets at a ratio of 6-16% body weight (four times a day, 08:30, 12:00, 16:00 and 19:30 h) for 10 weeks. The rearing water was exchanged every 3 days. Water quality was maintained within a standard range for *L. vannamei* during the experiment period

Sample collection

At the end of the feeding trial, all the shrimp in each tank were bulk-weighed and individual-weighed for calculation of growth parameters and survival (Fig. 3). Four shrimp per tank (thirty shrimp per dietary treatment) were randomly captured and placed in ice water for 5 min to anesthetize before hemolymph sampling. Hemolymph was withdrawn from the ventral sinus of each shrimp into a 1 mL syringe (25gauge needle) containing 400 µL of precooled (4°C) anticoagulant solution (Alsever's solution, sigma) (Fig. 4). The diluted hemolymph from each shrimp was kept and analyzed separately. About 500 µl anticoagulant-hemolymph was used to determine nitroblue-tetrazolium (NBT), total haemocyte count (THC) and hyaline cell count. The remaining anticoagulant-hemolymph mixture was centrifuged at 800 × g for 20 min at 4℃ and the supernatant was stored at -70℃ for immune related analyses. Feeding was stopped 24 h prior to weighing or blood sampling to minimize stress.

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Estimation of apparent digestibility coefficients

For estimation of apparent digestibility coefficient of the experimental diets, chromic oxide (Cr_2O_3) (Sigma) were included in the diets as an inert indicator at a concentration of 10 g kg^{-1} diet. Another set of shrimp were stocked into the 240 L capacity tanks at a density of 40-50 shrimp per tank. Faecal samples were collected three times a day from each tank (11:30, 15:00 and 18:30 hours). All feces were collected from each tank which were pooled and later frozen at -40 ℃ until analyses. Chromium oxide content of diet and feces samples were analyzed by the method described by Divakaran et al. (2002).

Analyses

Water quality parameters

During the 10 week growing period, the water quality parameters such as dissolved oxygen (Orion Star A216 Benchtop, Thermo Scientific) and pH (Seven Compact, METTLER TOLEDO) were monitored. NH_4^+ was analyzed by the method described by Verdouw et al. (1978).

Proximate composition analysis

Analysis of moisture, ash content and the muscle water retention rate were performed by the standard procedures (AOAC, 2005). Crude protein was measured by using automatic Kjeltec Analyzer Unit 2300 (FOSS, Sweden) and crude lipid was analyzed by the method described by Folch et al. (1957).

Immunological assays

The anti-protease activity was measured according to the method described by Ellis (1990), with slight modifications (Magnadóttir et al., 1999). Hemolymph superoxide

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dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 (Water Soluble Tetrazolium dye) substrate and xanthine oxidase using a SOD Assay Kit (Sigma, 19160) according to the manufacturer's instructions. GPx activity was assayed using kit (Biovision, Inc. California, USA). A turbidometric assay was used for determination of serum lysozyme level through the method described by Hultmark (1980) with slight modifications. Catalase activity was assayed using kit (Biovision, Inc. California, USA). Total antioxidant capacity (TAC) was determined using a commercial kit (Antioxidant assay kit, Sigma; CS0790). Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA, Sigma) using the method described by Hernández-López et al (1996). Oxidative radical production by phagocytes during respiratory burst was measured through NBT assay described by Zhang et al (2013).

Hematological parameters

Levels of cholesterol, triglyceride, glucose and total protein were determined by an automated blood analyzer (SLIM, SEAC Inc, Florence, Italy). The total numbers of haemocytes and hyaline cells within the measured hemolymph were measured with a haemocytometer using an inverted phase-contrast microscope (Leica DMIL, Leica Microsystems GmbH, Wetzlar, Germany).

Intestin and hepatopancreas morphology

Intestin and hepatopancreas samples were collected from two shrimp per tank and fixed in Bouin’s solution. The fixed tissues were dehydrated in ascending concentrations of alchol, cleared in toluene, embedded in paraffin and sectioned with a rotary microtome at 5μm. Slides were stained with Harris hematoxylin, 0.5% eosin, alcian blue (AB) at pH 2.5

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and periodic acid Schiff (PAS) for observation of the villus height and hepatopancreas tubules. Then, the slides were observed to identify hepatopancreas cells(Four cell types dominate the hepatopancreas tubules; namely E "embryonalzellen" or embryonic" cells, R "restzellen" cells, F "fibrillenzellen" or "fibrous" cells, and B "blasenzellen" cells) and measure villus height using the ImageJ 1.44 analysis software (Fig. 7).

Challenge test

At the end of the feeding trial, 8 shrimp from each tank (45 shrimp per treatment) were randomly selected and subjected to a bacterial challenge. *V. harveyi* was used as the pathogenic agent (provided by the Marine Microbiology Laboratory of Jeju National University). The shrimp was injected intramuscularly with *V. harveyi* suspension containing 2×10^5 CFU mL^{-1}. The pathogenic dose of bacterium had previously been determined in a preliminary test using shrimp of a similar size. After injection, the shrimp was distributed into 110 L acryl tanks, and their mortality was monitored and recorded for 23 days.

Statistical analysis

All experimental diets were assigned using a completely randomized design. Data were analyzed by one-way analysis of variance (ANOVA) in SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). When ANOVA identified differences among groups, the differences in mean values were compared using Tukey's HSD test at the 5% level of significance (P <0.05). Data are presented as mean ± SD. Percentage data were arcsine transformed before analysis.

3. Results

Growth performance, feed utilization and survival rate of shrimp fed seven experimental

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diets are shown in Table 6.3. FBW of shrimp fed SLP diet was significantly lower compared to other diets except for TuH-3 group. However, WG and PER of shrimp were significantly improved by DFS-4, SH-1 and TuH-1.5 diets compared to SLP diet. FCR of shrimp fed SLP diet was significant to other shrimp groups except for TuH-3 group. Therefore, feed efficiency was followed the same trend as observed in FBW. However, no significant affects were observed on survival of shrimp.

The results of non-specific immune response parameters are shown in Table 6.4. SOD activity of shrimp fed DFS-2 diet was significant to TuH-1.5 group. GPx activity of shrimp fed SH diets was significantly higher than SLP and DFS-4 groups. NBT activity of shrimp was significantly increased by SH-2 diet compared to that of shrimp fed control and DFS-2 diets. TAC was significant in shrimp fed SH-1 or TuH-3 diets compared to TuH-1.5 and DFS-2 groups. Catalase activity of shrimp fed SH-2 diet was significant to SLP, DFS-2, DFS-4 and SH-1 groups. However, no significant differences were observed in antiprotease , lysozyme and PO activities.

The results of hematological parameters are shown in Table 6.5. Hemolymph THC was significantly higher in SH-2, TuH-1.5 and TuH-3 groups compared to SLP group. Hyaline cell count was significantly lower in shrimp fed SLP diet compared to other groups. There was no significant differenced were observed in cholesterol, triglyceride, glucose and total protein levels of hemolymph. No significant differences were observed in whole body proxymate composition of shrimp by dietary treatments (Table 6.6).

Intestinal and hepatopancreas morphology of shrimp are shown in Table 6.7. No significant differences were observed in hepatopancreas histology. However, intestinal VL of shrimp was significantly different in DFS-4, SH-1, SH-2 and TuH-1.5 groups than control

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group. Apparent digestibility coefficient of protein, dry matter and amino acid are shown in table 6.8 and 6.9. Protein digestibility was significantly higher in diet containing hydrolysates than SLP group. However, the highest protein digestibility was shown in TuH-1.5 diet. Dry matter digestibility was significantly higher in DFS-2, SH-1, TuH-1.5 and TuH-3 diets compared to SLP diet. Amino acid digestibility was significant in TuH-1.5 diet than the other groups.

At the end of the challenge test, first mortality of shrimp injected with *V. harveyi* was observed after 2 days. Disease resistance against *V. harveyi* was significantly affected by the dietary treatments as the control group showed lowest survival rate. However, DFS-2and DFS-4 group showed higher survival rate compared to the other treatments (Figure 6.1).

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Table 6. 3. Growth performance and feed utilization of Pacific white shrimp fed the seven experimental diets for 52 days.

	FBW[2] (g)	WG[3] (%)	FCR[4]	PER[5]	Survival (%)
SLP	9.01±0.36[b]	8102±330[b]	1.57±0.07[a]	1.65±0.07[b]	82.4±5.4
DFS-2	10.07±0.4[a]	9001±333[ab]	1.41.±0.06[b]	1.81±0.08[ab]	84.8±11
DFS-4	10.31±0.3[a]	9328±37[a]	1.38±0.05[b]	1.85±0.06[a]	87.2±3.3
SH-1	10.37±0.32[a]	9320±383[a]	1.36±0.04[b]	1.88±0.06[a]	84.0±2.8
SH-2	10.07±0.47[a]	9004±388[ab]	1.41±0.07[b]	1.82±0.08[ab]	85.6±7.8
TuH-1.5	10.17±0.96[a]	9112±903[a]	1.40±0.13[b]	1.84±0.16[a]	80.8±3.3
TuH-3	9.80±0.37[ab]	8722±350[ab]	1.45±0.06[ab]	1.77±0.08[ab]	88.0±7.5

Values are mean of five replicates groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p<0.05$).

[1]IBW: initial body weight (g)

[2]FBW: final mean body weight (g)

[3]Weight gain (%) = 100 x (final mean body weight – initial mean body weight)/initial mean body weight

[4]Feed conversion ratio = dry feed fed (g) / wet weight gain (g)

[5]Protein efficiency ratio = wet weight gain /total protein given

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Table 6. 4. Non-specific immune parameters of Pacific white shrimp fed the seven experimental diets for 52 days.

	SLP	DFS-2	DFS-4	SH-1	SH-2	TuH-1.5	TuH-3
Antipro	24.2±3.3	27.6±3.7	24.1±3.7	26.0±2.6	25.7±4.1	24.0±1.9	24.9±3.5
SOD[2]	74.6±8.2[ab]	83.0±6.3[a]	79.4±4.8[ab]	77.8±6.7[ab]	78.3±6.8[ab]	72.5±5.4[b]	76.3±5.2[ab]
GPx[3]	30.2±1.2[b]	34.1±3.7[ab]	31.7±3.8[b]	36.9±3.0[a]	36.6±1.3[a]	33.2±4.1[ab]	32.1±4.4[ab]
Lysoz[4]	4.90±0.62	5.00±0.60	5.12±1.10	5.03±0.54	5.10±0.67	5.49±1.41	5.09±0.72
PO[5]	0.128±0.00	0.133±0.01	0.129±0.01	0.134±0.03	0.128±0.01	0.128±0.01	0.122±0.02
NBT[6]	2.43±0.3[b]	2.54±0.4[b]	2.93±0.3[ab]	2.80±0.5[ab]	3.07±0.2[a]	2.80±0.3[ab]	2.92±0.3[ab]
TAC[7]	2.45±0.19[ab]	2.33±0.07[b]	2.51±0.06[ab]	2.62±0.17[a]	2.51±0.11[ab]	2.31±0.14[b]	2.56±0.14[a]
Cat[8]	0.69±0.11[b]	0.74±0.20[b]	0.75±0.15[b]	0.76±0.17[b]	1.05±0.21[a]	0.96±0.15[ab]	0.82±0.29[ab]

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same raw are significantly different ($p < 0.05$).

[1] Antiprotease activity (% inhibition)

[2] Superoxide dismutase (% inhibition)

[3] Glutathione peroxidase activity (mU ml^{-1})

[4]Lysozyme activity (μg ml^{-1})

[5] Phenoloxidase activity (absorbance)

[6] Nitro blue tetrazolium; phagocytic activity (absorbance)

[7]Total antioxidant capacity (μmol ml^{-1})

[8]Catalase activity

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Table 6. 5. Hematological parameters of Pacific white shrimp fed the seven experimental diets for 52 days.

	Gluco[1]	Triglycer[2]	Total pro[3]	Cholest[4]	THC[5]	HC[6]
SLP	585±37	21.3±3.4	1.20±0.27	3.11±0.84	125±7.4[b]	62.3±3.9[b]
DFS-2	576±44	19.2±2.3	1.41±0.60	3.24±0.59	140±4.7[ab]	68.9±6.8[a]
DFS-4	594±13	22.1±3.2	1.52±0.41	3.49±0.81	137±3.0[ab]	69.6±4.3[a]
SH-1	590±24	17.4±2.5	1.46±0.33	4.04±0.84	139±7.9[ab]	68.4±2.7[a]
SH-2	576±33	19.9±3.7	1.46±0.31	4.08±0.77	143±3.7[a]	69.2±4.9[a]
TuH-1.5	582±21	22.7±2.4	1.31±0.26	3.75±1.63	146±15[a]	68.5±2.6[a]
TuH-3	594±19	20.9±2.5	1.53±0.23	3.36±0.88	149±6.0[a]	68.4±3.2[a]

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

1Glucose (mg dL^{-1})

2Triglyceride (mg dL^{-1})

3Total protein (g dL^{-1})

4Total cholesterol (mg dL^{-1})

[5]Total haemocyte count (10^5 cells ml^{-1})

[6]Hyaline cells count (10^5 cells ml^{-1})

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Table 6. 6. Whole body composition (%) Pacific white shrimp fed the seven experimental diets for 10 weeks.

	Moisture	Protein	Lipid	Ash
SLP	76.5±0.14	18.5±0.58	0.74±0.11	14.3±0.4
DFS-2	75.6±0.71	18.1±1.02	0.85±0.28	14.2±1.2
DFS-4	76.4±0.25	18.6±051	0.73±0.34	14.3±0.7
SH-1	76.0±0.32	18.3±1.06	0.59±0.14	14.3±2.1
SH-2	76.0±0.33	18.7±1.42	0.9±0.41	14.4±2.0
TuH-1.5	75.8±0.54	18.5±0.76	1.03±0.12	12.2±1.1
TuH-3	75.5±0.45	18.9±1.00	0.83±0.19	13.6±1.4

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

Table 6. 7. Intestinal morphology of Pacific white shrimp fed seven experimental diets for 52 days.

	Villus height, μm
SLP	70.9 ± 2.1^{b}
DFS-2	74.0 ± 6.0^{ab}
DFS-4	77.4 ± 2.8^{a}
SH-1	77.3 ± 3.0^{a}
SH-2	78.0 ± 4.7^{a}
TuH-1.5	77.3 ± 2.2^{a}
TuH-3	74.7 ± 12.4^{ab}

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

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Table 6. 8. Apparent digestibility coefficients (%, ADC) for dry matter and protein of the experimental diets for Pacific white shrimp.

	ADCp (%)[2]	ADCd (%)[1]
SLP	89.5±0.4^{d}	75.5±0.9^{b}
DFS-2	90.7±0.4^{bc}	77.4±0.9^{ab}
DFS-4	90.5±0.5^{bc}	78.3±1.2^{a}
SH-1	91.8±0.5^{b}	77.7±1.2^{a}
SH-2	91.2±0.8^{bc}	77.3±2.1^{ab}
TuH-1.5	95.2±0.6^{a}	77.9±1.8^{a}
TuH-3	90.8±0.4^{c}	77.5±1.0^{a}

Values are mean of five replicate groups and presented as mean ± S.D. Values with different superscripts in the same column are significantly different ($p < 0.05$).

[1] Apparent digestibility coefficient of dry matter

[2] Apparent digestibility coefficient of protein

Table 6. 9. Apparent digestibility coefficients (%, ADC) for amino acid of the experimental diets for Pacific white shrimp.

	SLP	DFS-2	DFS-4	SH-1	SH-2	TuH-1.5	TuH-3
Aspartic Acid	90.0±0.8	91.2±0.8	90.1±0.5	92.5±0.2	91.9±0.8	94.2±0.4	90.4±0.3
Threonine	86.3±0.9	87.7±0.8	86.7±0.6	89.7±0.4	89.7±1.0	92.1±0.5	87.4±0.6
Serin	90.0±0.4	91.8±0.6	90.2±0.4	92.3±0.4	92.9±0.7	94.1±0.6	91.6±0.4
Glutamic Acid	94.5±0.4	94.3±0.3	94.1±0.3	95.6±0.1	94.9±0.5	96.5±0.2	94.4±0.2
Proline	92.4±0.5	93.5±0.4	93.0±0.4	94.0±0.3	93.4±0.4	95.2±0.5	93.5±0.4
Glycine	86.5±1.3	88.4±0.6	88.2±0.6	90.1±0.3	89.0±1.1	91.2±0.8	88.3±0.5
Alanine	85.7±1.4	86.6±0.6	86.7±0.7	89.4±0.3	88.3±1.2	91.6±0.6	86.5±0.6
Valine	88.2±1.2	88.7±0.6	88.3±0.7	91.3±0.2	89.8±1.1	93.4±0.4	89.4±0.5
Isoleucine	90.5±1.1	90.5±0.5	90.2±0.6	92.8±0.2	91.5±0.8	94.8±0.2	91.2±0.4
Leucine	91.0±0.9	89.8±0.6	91.4±0.5	93.2±0.3	92.1±0.8	95.1±0.2	91.8±0.3
Tyrosine	89.3±0.5	90.0±1.0	89.8±0.4	91.7±0.3	92.2±1.7	94.7±0.4	91.4±0.5
Phenylalanine	90.9±0.8	91.5±0.5	90.9±0.5	93.3±0.2	92.2±0.8	94.9±0.3	92.0±0.4
Histidine	85.4±0.7	90.1±0.9	90.8±0.4	88.2±0.7	89.5±0.7	90.0±2.3	90.2±0.9
Lysine	92.5±0.3	93.2±0.8	93.5±0.5	94.1±0.6	93.4±0.9	95.9±0.1	93.2±0.6
Arginine	93.1±0.8	94.1±0.3	94.0±0.5	95.2±0.1	94.7±0.6	96.0±0.1	94.3±0.3

Values are mean of four replicate groups and presented as mean ± S.D. Values with different superscripts in the same raw are significantly different ($p < 0.05$).

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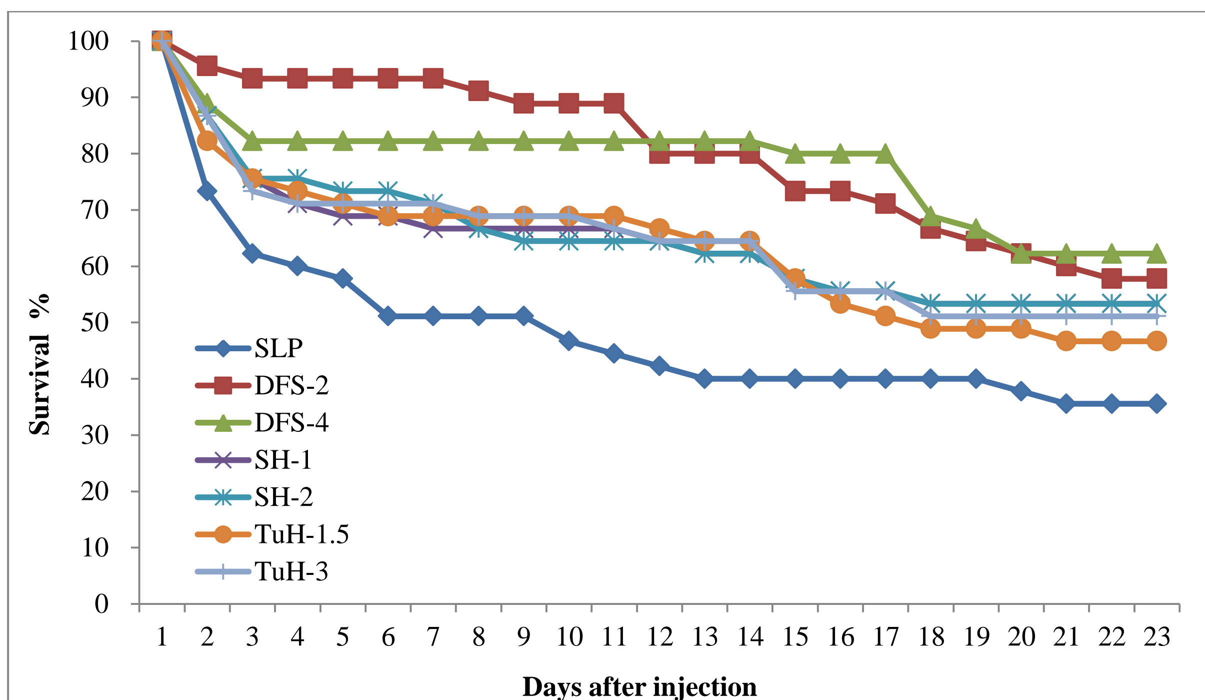

Figure 6. 1. Survival rate of Pacific white shrimp fed the seven experimental diets after challenge with *Vibrio harveyi*.

4. Discussion

Growth performance and feed utilization of shrimp were significantly improved by feeding diet containing DFS, SH and TuH in the present study. The feeding trial was conducted under 27-30°C water temperature which is in the range of standard for PWS to maintain optimum growth (Wyban et al., 1995). Although PWS is an omnivorous species, inclusion of marine protein sources in their diet is required for a better feed utilization in commercial aquaculture as higher level of plant protein in diets resulted in lower growth rates (Dominy, 1990; Amaya et al., 2007; Suárez et al., 2009; Bauer et al., 2012). Growth and feed utilization of fish and shrimp can be improved by dietary supplementation of ingredient produced by squid processing by-products. Growth of Japanese seabass (*Lateolabrax*

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japonicas) was improved by dietary squid viscera meal supplementation compared to a high FM diet (Mai et al., 2006). Growth performance of PWS were increased by dietary squid meal supplementation up to 9% and decreased when 15% of total protein was supplemented with squid meal in the diet (Córdova-Murueta et al., 2002). SH has been evaluated in diet for several fish and shrimp species and positive effects were elucidated on growth, immunity, digestibility, palatability and health of fish (Plascencia-Jatomea at el., 2002; Leal at el., 2010; Koshravi et al., 2015a; Koshravi et al., 2015b; Khoshravi et al., 2017; Leduc at el., 2018). According to Nguyen et al. (2012), growth performance of shrimp was improved by dietary tuna head hydrolysate supplementation. Hernández et al. (2011) identified that tuna by-product hydrolysates provide a palatability to shrimp feed while improving protein digestibility and amino acid profile of feed. It is reported that fish soluble supplementation to red drum (*Sciuenops ocellatus*) diets was beneficial to improve growth and feed utilization (McGoogan and Gatlin, 1997). Also, fish soluble is recommended to use in high plant protein diets for kuruma shrimp (*Marsupenaeus japonicas*) to replace FM and improve growth performance. Therefore, it is reasonable to increase growth performance of shrimp according to above evidences. Eventually, we couldn't observe any significant changes by the different levels of DFS or SH indicating that these levels are matching the requirement of PWS. However, due to the tuna hydrolysate inclusion up to 3% in diet for PWS seems to reduce growth performance and feed utilization, further studies are required to estimate proper inclusion level of these three ingredients.

Non-specific immune parameters were influenced by dietary DFS, SH or TuH supplementation in different levels to PWS although levels of three products did not significantly change the immune responses except for the TAC of TuH-3 group and catalase

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activity of SH-2 group. Immunostimulatory effects of different hydrolysates products have been revealed over past decade (Huang et al., 2014; Chalamaiah et al., 2015; Karnjanapratum et al., 2016; Chalamaiah et al., 2018). It was reported that innate immunity of seabass and Atlantic halibut was enhanced by dietary supplementation of hydrolysates from pollock by-product (Liang et al., 2006; Hermannsdottir et al., 2009). Immunity of fish and shrimp can be improved by feeding diets containing hydrolyzed shrimp protein (Plascencia-Jatomea at el., 2002; Leal at el., 2010; Koshravi et al., 2015a; Koshravi et al., 2015b; Khoshravi et al., 2017; Leduc at el., 2018). Tuna frame protein hydrolysates is a beneficial ingredient which contain peptides to act as nutraceuticals and pharmaceuticals against diseases related to hypertension (Lee et al., 2010). Similarly, hydrolysates from bigeye tuna (*Thunnus obesus*) dark muscle also beneficial against hypertension (Qian et al., 2007). Therefore, these evidences justify the improvement of immunity by tested products. TAC of TuH-3 group and catalase activity of SH-2 group were significantly higher compared to TuH-1.5 and SH-1 groups respectively. Both TuH and SH contain peptides generated during the process of hydrolysis. With the increase of hydrolysates in diets, high amount of immunomodulatory peptides might be supplied to shrimp resulting boosted immunity.

In the present study, ADC of dry matter and protein of DFS, SH and TuH diets were significantly higher compared to SLP diet. Yang et al. (2009) observed that dry matter and energy digestibilities of shrimp byproduct were lower due to the quantity and chemical composition of the chitin. In this study, we observed higher DM and protein digestibility of SH diets. Therefore, it provide evident to prove that the quality of shrimp byproducts was improved during the process of hydrolysis. Hernández et al. (2011) reported that tuna byproduct hydrolysates improved the protein digestibility of PWS. Digestibility of plant

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based ingredients is lower in shrimp compared to animal protein (Yang et al., 2009). Factors unrelated to diet formulation, such as environmental conditions in the production system, feeding practices and manufacturing techniques, also affect diet digestibility under practical culture conditions (Gabbott and Forster,1971; Brunson et al., 2003). Therefore, further studies are required to evaluate effects of these ingredients on diet digestibility of shrimp.

In conclusion, dietary supplementation of 2% SH and 1.5-3% TuH seems to improve growth and immunity of PWS.

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JEJU NATIONAL UNIVERSITY LIBRARY

References

Ahn, C. B., Je, J. Y. and Cho, Y. S. (2012) 'Antioxidant and anti-inflammatory peptide fraction from salmon byproduct protein hydrolysates by peptic hydrolysis', *Food Research International*, 49(1), pp. 92–98. doi: 10.1016/j.foodres.2012.08.002.

Ahn, C. B., Kim, J. G. and Je, J. Y. (2014) 'Purification and antioxidant properties of octapeptide from salmon byproduct protein hydrolysate by gastrointestinal digestion', *Food Chemistry*, 147, pp. 78–83. doi: 10.1016/j.foodchem.2013.09.136.

Aksnes, A. *et al.* (2006) 'Size-fractionated fish hydrolysate as feed ingredient for rainbow trout (Oncorhynchus mykiss) fed high plant protein diets. I: Growth, growth regulation and feed utilization', *Aquaculture*, 261(1), pp. 305–317. doi: 10.1016/j.aquaculture.2006.07.025.

Aksnes, A. and Mundheim, H. (1997) 'The impact of raw material freshness and processing temperature for fish meal on growth, feed efficiency and chemical composition of atlantic halibut (Hippoglossus hippoglossus)', *Aquaculture*. Elsevier, 149(1–2), pp. 87–106. doi: 10.1016/S0044-8486(96)01438-X.

Alam, M. S. *et al.* (2005) 'Supplemental effects of coated methionine and/or lysine to soy protein isolate diet for juvenile kuruma shrimp, Marsupenaeus japonicus', in *Aquaculture*, pp. 13–19. doi: 10.1016/j.aquaculture.2005.04.015.

Amaya, E., Davis, D. A. and Rouse, D. B. (2007) 'Alternative diets for the Pacific white shrimp Litopenaeus vannamei', *Aquaculture*, 262(2–4), pp. 419–425. doi: 10.1016/j.aquaculture.2006.11.001.

Bauer, W. *et al.* (2012) 'Substitution of fishmeal with microbial floc meal and soy protein concentrate in diets for the pacific white shrimp Litopenaeus vannamei', *Aquaculture*, 342–343(1), pp. 112–116. doi: 10.1016/j.aquaculture.2012.02.023.

Biswas, A. K. *et al.* (2005) 'Photoperiod influences the growth, food intake, feed efficiency and digestibility of red sea bream (Pagrus major)', *Aquaculture*. Elsevier, 250(3–4), pp. 666–673. doi: 10.1016/J.AQUACULTURE.2005.04.047.

Bjorndal, B. *et al.* (2012) 'Krill powder increases liver lipid catabolism and reduces glucose mobilization in tumor necrosis factor-alpha transgenic mice fed a high-fat diet', *Metabolism: Clinical and Experimental*. W.B. Saunders, 61(10), pp. 1461–1472. doi: 10.1016/j.metabol.2012.03.012.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Bonaldo, A. *et al.* (2011) 'Increasing dietary plant proteins affects growth performance and ammonia excretion but not digestibility and gut histology in turbot (Psetta maxima) juveniles', *Aquaculture*, 318(1–2), pp. 101–108. doi: 10.1016/j.aquaculture.2011.05.003.

Brunson, J. F., Romaire, R. P. and Reigh, R. C. (1997a) 'Apparent digestibility of selected ingredients in diets for white shrimp Penaeus setiferus L.', *Aquaculture Nutrition*, 3(1), pp. 9–16. doi: 10.1046/j.1365-2095.1997.00068.x.

Brunson, J. F., Romaire, R. P. and Reigh, R. C. (1997b) 'Apparent digestibility of selected ingredients in diets for white shrimp Penaeus setiferus L', *Aquaculture Nutrition*. Blackwell Publishing Ltd, 3(1), pp. 9–16. doi: 10.1046/j.1365-2095.1997.00068.x.

Bui, H. T. D. *et al.* (2014) 'Growth performance, feed utilization, innate immunity, digestibility and disease resistance of juvenile red seabream (Pagrus major) fed diets supplemented with protein hydrolysates', *Aquaculture*. Elsevier, 418–419, pp. 11–16. doi: 10.1016/J.AQUACULTURE.2013.09.046.

Bulbul, M. *et al.* (2015) 'Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for kuruma shrimp, Marsupenaeus japonicus', *Aquaculture*. Elsevier, 438, pp. 98–104. doi: 10.1016/j.aquaculture.2015.01.007.

Cahú, T. B. *et al.* (2012) 'Recovery of protein, chitin, carotenoids and glycosaminoglycans from Pacific white shrimp (Litopenaeus vannamei) processing waste', *Process Biochemistry*, 47(4), pp. 570–577. doi: 10.1016/j.procbio.2011.12.012.

Carvalho, M. *et al.* (2018a) 'Dietary requirement for n-3 long-chain polyunsaturated fatty acids for fast growth of meagre (Argyrosomus regius, Asso 1801) fingerlings', *Aquaculture*. Elsevier, 488, pp. 105–113. doi: 10.1016/J.AQUACULTURE.2018.01.028.

Carvalho, M. *et al.* (2018b) 'Dietary requirement for n-3 long-chain polyunsaturated fatty acids for fast growth of meagre (Argyrosomus regius, Asso 1801) fingerlings', *Aquaculture*. Elsevier, 488, pp. 105–113. doi: 10.1016/J.AQUACULTURE.2018.01.028.

Chalamaiah, M. *et al.* (2014) 'Immunomodulatory effects of protein hydrolysates from rohu (Labeo rohita) egg (roe) in BALB/c mice', *Food Research International*. Elsevier Ltd, 62, pp. 1054–1061. doi: 10.1016/j.foodres.2014.05.050.

Chalamaiah, M. *et al.* (2015) 'Chemical composition and immunomodulatory effects of enzymatic protein hydrolysates from common carp (Cyprinus carpio) egg', *Nutrition*. Elsevier, 31(2), pp.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

388–398. doi: 10.1016/J.NUT.2014.08.006.

Chalamaiah, M., Yu, W. and Wu, J. (2018) ‘Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review’, *Food Chemistry*. Elsevier, 245, pp. 205–222. doi: 10.1016/J.FOODCHEM.2017.10.087.

Cheng, M. L. *et al.* (2015) ‘Anti-inflammatory peptides from enzymatic hydrolysates of tuna cooking juice’, *Food and Agricultural Immunology*. Taylor and Francis Ltd., 26(6), pp. 770–781. doi: 10.1080/09540105.2015.1036352.

Cho, J.-H. *et al.* (2018) ‘Periodic changes in the growth performance and biochemical composition of juvenile red sea bream Pagrus major fed non-heated and heated squid and krill meal-based diets’, *Fisheries Science*. Springer Japan, 84(4), pp. 699–713. doi: 10.1007/s12562-018-1205-6.

Córdova-Murueta, J. H. and García-Carreño, F. L. (2002) ‘Nutritive value of squid and hydrolyzed protein supplement in shrimp feed’, *Aquaculture*, 210(1–4), pp. 371–384. doi: 10.1016/S0044-8486(02)00011-X.

Cruz-Suárez, L. E. *et al.* (2007) ‘Replacement of fish meal with poultry by-product meal in practical diets for Litopenaeus vannamei, and digestibility of the tested ingredients and diets’, *Aquaculture*, 272(1–4), pp. 466–476. doi: 10.1016/j.aquaculture.2007.04.084.

Cruz-Suárez, L. E., Guillaume, J. and Van Wormhoudt, A. (1989) ‘Effect of squid extracts on time course appearance of glucose and free amino acids in haemolymph in Penaeus japonicus after feeding: Preliminary results’, *Aquaculture*, 76(1–2), pp. 57–65. doi: 10.1016/0044-8486(89)90251-2.

Cuesta, A., Meseguer, J. and Esteban, M. Á. (2008) ‘The antimicrobial peptide hepcidin exerts an important role in the innate immunity against bacteria in the bony fish gilthead seabream’, *Molecular Immunology*, 45(8), pp. 2333–2342. doi: 10.1016/j.molimm.2007.11.007.

Dabrowski, K. *et al.* (2010) ‘Effects of protein-, peptide- and free amino acid-based diets in fish nutrition’, *Aquaculture Research*, 41(5), pp. 668–683. doi: 10.1111/j.1365-2109.2010.02490.x.

Derby, C. D. and Sorensen, P. W. (2008) ‘Neural processing, perception, and behavioral responses to natural chemical stimuli by fish and crustaceans’, *Journal of Chemical Ecology*, pp. 898–914. doi: 10.1007/s10886-008-9489-0.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Van Doan, H., Doolgindachbaporn, S. and Suksri, A. (2014) 'Effects of low molecular weight agar and Lactobacillus plantarum on growth performance, immunity, and disease resistance of basa fish (Pangasius bocourti, Sauvage 1880)', *Fish and Shellfish Immunology*. Academic Press, 41(2), pp. 340–345. doi: 10.1016/j.fsi.2014.09.015.

Dossou, S. *et al.* (2018) 'Growth performance, blood health, antioxidant status and immune response in red sea bream (Pagrus major) fed Aspergillus oryzae fermented rapeseed meal (RM-Koji)', *Fish & Shellfish Immunology*. Academic Press, 75, pp. 253–262. doi: 10.1016/J.FSI.2018.01.032.

Feeding attraction activities of food attractarnts for 3 species of fishes - Abstract - Europe PMC (no date). Available at: https://europepmc.org/abstract/cba/575116 (Accessed: 1 November 2019).

Floreto, E. A. T., Brown, P. B. and Bayer, R. C. (2001) 'The effects of krill hydrolysate-supplemented soya-bean based diets on the growth, colouration, amino and fatty acid profiles of juvenile American lobster, Homarus americanus', *Aquaculture Nutrition*, 7(1), pp. 33–43. doi: 10.1046/j.1365-2095.2001.00154.x.

'full-text' (no date).

Gabbott, P. A. and Forster, J. R. M. (1971) 'The Assimilation Of Nutrients From Compounded Diets By The Prawns Palaemon Serratus And Pandalus Platyceros', *Journal of the Marine Biological Association of the United Kingdom*, 51(4), pp. 943–961. doi: 10.1017/S0025315400018075.

Gaber, M. M. A. (2007a) 'The Effect of Different Levels of Krill Meal Supplementation of Soybean-based Diets on Feed Intake, Digestibility, and Chemical Composition of Juvenile Nile Tilapia Oreochromis niloticus, L', *Journal of the World Aquaculture Society*. John Wiley & Sons, Ltd (10.1111), 36(3), pp. 346–353. doi: 10.1111/j.1749-7345.2005.tb00338.x.

Gaber, M. M. A. (2007b) 'The Effect of Different Levels of Krill Meal Supplementation of Soybean-based Diets on Feed Intake, Digestibility, and Chemical Composition of Juvenile Nile Tilapia Oreochromis niloticus, L', *Journal of the World Aquaculture Society*. John Wiley & Sons, Ltd (10.1111), 36(3), pp. 346–353. doi: 10.1111/j.1749-7345.2005.tb00338.x.

Garzade Yta, A., Rouse, D. B. and Davis, D. A. (2004) 'Influence of Nursery Period on the Growth and Survival of Litopenaeus vannamei Under Pond Production Conditions', *Journal of the World Aquaculture Society*. Wiley, 35(3), pp. 357–365. doi: 10.1111/j.1749-

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

7345.2004.tb00099.x.

Gisbert, E. *et al.* (2018) 'Diets containing shrimp protein hydrolysates provided protection to European sea bass (Dicentrarchus labrax) affected by a Vibrio pelagius natural infection outbreak', *Aquaculture*. Elsevier B.V., 495, pp. 136–143. doi: 10.1016/j.aquaculture.2018.04.051.

Gómez-Requeni, P. *et al.* (2004) 'Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (Sparus aurata)', *Aquaculture*, 232(1–4), pp. 493–510. doi: 10.1016/S0044-8486(03)00532-5.

Gomez, D., Sunyer, J. O. and Salinas, I. (2013) 'The mucosal immune system of fish: The evolution of tolerating commensals while fighting pathogens', *Fish & Shellfish Immunology*. Academic Press, 35(6), pp. 1729–1739. doi: 10.1016/J.FSI.2013.09.032.

Goto, T. *et al.* (2001a) 'Studies on the green liver in cultured red sea bream fed low level and non-fish meal diets: Relationship between hepatic taurine and biliverdin levels', *Fisheries Science*. John Wiley & Sons, Ltd (10.1111), 67(1), pp. 58–63. doi: 10.1046/j.1444-2906.2001.00199.x.

Goto, T. *et al.* (2001b) 'Studies on the green liver in cultured red sea bream fed low level and non-fish meal diets: Relationship between hepatic taurine and biliverdin levels', *Fisheries Science*, 67(1), pp. 58–63. doi: 10.1046/j.1444-2906.2001.00199.x.

Guerard, F., Guimas, L. and Binet, A. (2002) 'Production of tuna waste hydrolysates by a commercial neutral protease preparation', *Journal of Molecular Catalysis B: Enzymatic*, 19(20), pp. 489–498. doi: 10.1016/S1381-1177(02)00203-5.

Gunathilaka, G. L. B. E. *et al.* (2019) 'Effects of taurine supplementation in low fish meal diets for red seabream (Pagrus major) in low water temperature season', *Fisheries and Aquatic Sciences*. BioMed Central Ltd., 22(1). doi: 10.1186/s41240-019-0138-z.

Hansen, J. Ø. *et al.* (2010) 'High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (Salmo salar)', *Aquaculture*. Elsevier, 310(1–2), pp. 164–172. doi: 10.1016/j.aquaculture.2010.10.003.

Hardy, R. W. (2008) 'Alternative marine sources of fish feed and farmed fish quality', in *Improving Farmed Fish Quality and Safety*. Woodhead Publishing, pp. 328–342. doi: 10.1533/9781845694920.2.328.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Hatanaka, A. *et al.* (2009) 'Isolation and identification of antihypertensive peptides from antarctic krill tail meat hydrolysate', *Journal of Food Science*, 74(4). doi: 10.1111/j.1750-3841.2009.01138.x.

Hatlen, B. *et al.* (2017) 'The effect of low inclusion levels of Antarctic krill (Euphausia superba) meal on growth performance, apparent digestibility and slaughter quality of Atlantic salmon (Salmo salar)', *Aquaculture Nutrition*. John Wiley & Sons, Ltd (10.1111), 23(4), pp. 721–729. doi: 10.1111/anu.12439.

Hermannsdottir, R. *et al.* (2009) 'Analysis of effects induced by a pollock protein hydrolysate on early development, innate immunity and the bacterial community structure of first feeding of Atlantic halibut (Hippoglossus hippoglossus L.) larvae', *Fish & Shellfish Immunology*. Academic Press, 27(5), pp. 595–602. doi: 10.1016/J.FSI.2009.05.007.

Hernández, C. *et al.* (2008) 'Partial replacement of fish meal by porcine meat meal in practical diets for Pacific white shrimp (Litopenaeus vannamei)', *Aquaculture*, 277(3–4), pp. 244–250. doi: 10.1016/j.aquaculture.2008.02.016.

Hernández, C. *et al.* (2011) 'Enhancement of shrimp Litopenaeus vannamei diets based on terrestrial protein sources via the inclusion of tuna by-product protein hydrolysates', *Aquaculture*, 317(1–4), pp. 117–123. doi: 10.1016/j.aquaculture.2011.03.041.

Herpandi *et al.* (2011) 'The Tuna Fishing Industry: A New Outlook on Fish Protein Hydrolysates', *Comprehensive Reviews in Food Science and Food Safety*, 10(4), pp. 195–207. doi: 10.1111/j.1541-4337.2011.00155.x.

HEVROY, E. M. *et al.* (2005) 'Nutrient utilization in Atlantic salmon (Salmo salar L.) fed increased levels of fish protein hydrolysate during a period of fast growth', *Aquaculture Nutrition*, 11(4), pp. 301–313. doi: 10.1111/j.1365-2095.2005.00357.x.

Hikima, S. *et al.* (2003) 'Characterization and function of kuruma shrimp lysozyme possessing lytic activity against Vibrio species', *Gene*. Elsevier, 316(1–2), pp. 187–195. doi: 10.1016/S0378-1119(03)00761-3.

Hoseinifar, S. H., Sun, Y.-Z. and Caipang, C. M. (2017) 'Short-chain fatty acids as feed supplements for sustainable aquaculture: an updated view', *Aquaculture Research*. John Wiley & Sons, Ltd (10.1111), 48(4), pp. 1380–1391. doi: 10.1111/are.13239.

Hou, H. *et al.* (2012) 'Purification and identification of immunomodulating peptides from enzymatic

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

hydrolysates of Alaska pollock frame', *Food Chemistry*, 134(2), pp. 821–828. doi: 10.1016/j.foodchem.2012.02.186.

Hsu, K. C. (2010) 'Purification of antioxidative peptides prepared from enzymatic hydrolysates of tuna dark muscle by-product', *Food Chemistry*, 122(1), pp. 42–48. doi: 10.1016/j.foodchem.2010.02.013.

Huang, D. *et al.* (2014) 'Immunostimulatory Activity of Protein Hydrolysate from Oviductus Ranae on Macrophage *In Vitro*', *Evidence-Based Complementary and Alternative Medicine*. Hindawi, 2014, pp. 1–11. doi: 10.1155/2014/180234.

Huang, G. R., Zhao, J. and Jiang, J. X. (2011) 'Effect of defatting and enzyme type on antioxidative activity of shrimp processing byproducts hydrolysate', *Food Science and Biotechnology*. The Korean Society of Food Science and Technology, 20(3), pp. 651–657. doi: 10.1007/s10068-011-0092-8.

Israel. Maḥlaḳah le-dayig., J.-J. *et al.* (1987) *Bamidgeh.* State of Israel, Ministry of Agriculture, Fisheries Dept. Available at: http://evols.library.manoa.hawaii.edu/handle/10524/57050 (Accessed: 12 August 2019).

Je, J. Y. *et al.* (2007) 'Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis', *Process Biochemistry*, 42(5), pp. 840–846. doi: 10.1016/j.procbio.2007.02.006.

Je, J. Y. *et al.* (2009) 'Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis', *Food Research International*, 42(9), pp. 1266–1272. doi: 10.1016/j.foodres.2009.06.013.

Jollès, P. and Jollès, J. (1984) 'What's new in lysozyme research? - Always a model system, today as yesterday', *Molecular and Cellular Biochemistry*. Kluwer Academic Publishers, pp. 165–189. doi: 10.1007/BF00285225.

Kader, Md. Abdul *et al.* (2010) 'Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, Pagrus major', *Aquaculture*. Elsevier, 308(3–4), pp. 136–144. doi: 10.1016/J.AQUACULTURE.2010.07.037.

Kader, Md Abdul *et al.* (2010) 'Supplemental effects of some crude ingredients in improving nutritive values of low fishmeal diets for red sea bream, Pagrus major', *Aquaculture*, 308(3–4), pp. 136–144. doi: 10.1016/j.aquaculture.2010.07.037.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Kader, M. A. *et al.* (2011) 'Growth, nutrient utilization, oxidative condition, and element composition of juvenile red sea bream Pagrus major fed with fermented soybean meal and scallop by-product blend as fishmeal replacement', *Fisheries Science*, 77(1), pp. 119–128. doi: 10.1007/s12562-010-0312-9.

Kader, Md. Abdul *et al.* (2012) 'Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, Pagrus major', *Aquaculture*. Elsevier, 350–353, pp. 109–116. doi: 10.1016/j.aquaculture.2012.04.009.

Kader, Md Abdul *et al.* (2012) 'Effect of complete replacement of fishmeal by dehulled soybean meal with crude attractants supplementation in diets for red sea bream, Pagrus major', *Aquaculture*, 350–353, pp. 109–116. doi: 10.1016/j.aquaculture.2012.04.009.

Kader, M. A. and Koshio, S. (2012) 'Effect of composite mixture of seafood by-products and soybean proteins in replacement of fishmeal on the performance of red sea bream, Pagrus major', *Aquaculture*. Elsevier, 368–369, pp. 95–102. doi: 10.1016/J.AQUACULTURE.2012.09.014.

Karnjanapratum, S. *et al.* (2016) 'Antioxidant, immunomodulatory and antiproliferative effects of gelatin hydrolysate from unicorn leatherjacket skin', *Journal of the Science of Food and Agriculture*. John Wiley & Sons, Ltd, 96(9), pp. 3220–3226. doi: 10.1002/jsfa.7504.

Kaushik, S. J. and Seiliez, I. (2010) 'Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs', *Aquaculture Research*, 41(3), pp. 322–332. doi: 10.1111/j.1365-2109.2009.02174.x.

Ketterer, B. (2001) 'A bird's eye view of the glutathione transferase field', *Chemico-Biological Interactions*, 138(1), pp. 27–42. doi: 10.1016/S0009-2797(01)00277-0.

Khosravi, S., Bui, H. T. D., *et al.* (2015) 'Dietary supplementation of marine protein hydrolysates in fish-meal based diets for red sea bream (Pagrus major) and olive flounder (Paralichthys olivaceus)', *Aquaculture*. Elsevier, 435, pp. 371–376. doi: 10.1016/j.aquaculture.2014.10.019.

Khosravi, S., Rahimnejad, S., Herault, M., Fournier, V., Lee, C.-R., *et al.* (2015) 'Effects of protein hydrolysates supplementation in low fish meal diets on growth performance, innate immunity and disease resistance of red sea bream Pagrus major', *Fish & Shellfish Immunology*. Academic Press, 45(2), pp. 858–868. doi: 10.1016/J.FSI.2015.05.039.

Khosravi, S., Rahimnejad, S., Herault, M., Fournier, V., Lee, C. R., *et al.* (2015) 'Effects of protein hydrolysates supplementation in low fish meal diets on growth performance, innate immunity

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

and disease resistance of red sea bream Pagrus major', *Fish and Shellfish Immunology*. Academic Press, 45(2), pp. 858–868. doi: 10.1016/j.fsi.2015.05.039.

Khosravi, S. *et al.* (2018) 'Supplementation of Protein Hydrolysates to a Low-fishmeal Diet Improves Growth and Health Status of Juvenile Olive Flounder, *Paralichthys olivaceus*', *Journal of the World Aquaculture Society*, 49(5), pp. 897–911. doi: 10.1111/jwas.12436.

Kim, D. H., Lipton, D. and Choi, J. Y. (2012) 'Analyzing the economic performance of the red sea bream Pagrus major offshore aquaculture production system in Korea', *Fisheries Science*. Springer Japan, 78(6), pp. 1337–1342. doi: 10.1007/s12562-012-0540-2.

Kissil, G. W. *et al.* (2000) 'Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead seabream Sparus aurata L.', *Aquaculture Research*, 31(7), pp. 595–601. doi: 10.1046/j.1365-2109.2000.00477.x.

Kleekayai, T. *et al.* (2015) 'Extraction of antioxidant and ACE inhibitory peptides from Thai traditional fermented shrimp pastes', *Food Chemistry*. Elsevier, 176, pp. 441–447. doi: 10.1016/J.FOODCHEM.2014.12.026.

Klotz, M. G., Klassen, G. R. and Loewen, P. C. (1997) 'Phylogenetic relationships among prokaryotic and eukaryotic catalases', *Molecular Biology and Evolution*, 14(9), pp. 951–958. doi: 10.1093/oxfordjournals.molbev.a025838.

Kolakowski, E. *et al.* (1980) 'Application of partial autoproteolysis to extraction of protein from Antarctic krill (Euphasia superba) Part 1. Effect of pH on protein extraction intensity', *Food / Nahrung*, 24(6), pp. 499–506. doi: 10.1002/food.19800240603.

Kolakowski, E., Bligh, L. G.-S. science and technology. E. by E. and 1992, undefined (no date) 'Optimization of autoproteolysis to obtain an edible product 'precipitate'from Antarctic krill (Euphausia superba Dana)'.

Kolkovski, S., Czesny, S. and Dabrowski, K. (2000) 'Use of krill hydrolysate as a feed attractant for fish larvae and juveniles', *Journal of the World Aquaculture Society*. World Aquaculture Society, 31(1), pp. 81–88. doi: 10.1111/j.1749-7345.2000.tb00701.x.

Kondo, F. *et al.* (2017) 'Effect of the squid viscera hydrolysate on growth performance and digestion in the red sea bream Pagrus major', *Fish Physiology and Biochemistry*. Springer Netherlands, 43(6), pp. 1543–1555. doi: 10.1007/s10695-017-0391-y.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Kotzamanis, Y. P. *et al.* (2007) 'Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to Vibrio anguillarum in European sea bass (Dicentrarchus labrax) larvae', *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 147(1), pp. 205–214. doi: 10.1016/j.cbpa.2006.12.037.

Krogdahl, A., Bakke-McKellep, A. M. and Baeverfjord, G. (2003) 'Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (Salmo salar L.)', *Aquaculture Nutrition*, 9(6), pp. 361–371. doi: 10.1046/j.1365-2095.2003.00264.x.

Leal, A. L. G. *et al.* (2010a) 'Use of shrimp protein hydrolysate in Nile tilapia (Oreochromis niloticus, L.) feeds', *Aquaculture International*. Springer Netherlands, 18(4), pp. 635–646. doi: 10.1007/s10499-009-9284-0.

Leal, A. L. G. *et al.* (2010b) 'Use of shrimp protein hydrolysate in Nile tilapia (Oreochromis niloticus, L.) feeds', *Aquaculture International*. Springer Netherlands, 18(4), pp. 635–646. doi: 10.1007/s10499-009-9284-0.

Leduc, A. *et al.* (2018) 'Dietary aquaculture by-product hydrolysates: impact on the transcriptomic response of the intestinal mucosa of European seabass (Dicentrarchus labrax) fed low fish meal diets', *BMC Genomics*. BioMed Central, 19(1), p. 396. doi: 10.1186/s12864-018-4780-0.

Lee, S. H., Qian, Z. J. and Kim, S. K. (2010) 'A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats', *Food Chemistry*, 118(1), pp. 96–102. doi: 10.1016/j.foodchem.2009.04.086.

Li, F. *et al.* (2018a) 'Effects of dietary supplementation with algal astaxanthin on growth, pigmentation, and antioxidant capacity of the blood parrot (Cichlasoma citrinellum × Cichlasoma synspilum)', *Journal of Oceanology and Limnology*. Science Press, 36(5), pp. 1851–1859. doi: 10.1007/s00343-019-7172-7.

Li, F. *et al.* (2018b) 'Effects of dietary supplementation with algal astaxanthin on growth, pigmentation, and antioxidant capacity of the blood parrot (Cichlasoma citrinellum × Cichlasoma synspilum)', *Journal of Oceanology and Limnology*. Science Press, 36(5), pp. 1851–1859. doi: 10.1007/s00343-019-7172-7.

Li, M. *et al.* (2014) 'Comparison effect of dietary astaxanthin and Haematococcus pluvialis on growth

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

performance, antioxidant status and immune response of large yellow croaker Pseudosciaena crocea', *Aquaculture*. Elsevier, 434, pp. 227–232. doi: 10.1016/J.AQUACULTURE.2014.08.022.

Li, M. *et al.* (2016) 'Effects of dietary taurine on growth, immunity and hyperammonemia in juvenile yellow catfish Pelteobagrus fulvidraco fed all-plant protein diets', *Aquaculture*. Elsevier, 450, pp. 349–355. doi: 10.1016/j.aquaculture.2015.08.013.

Liang, M. *et al.* (2006a) 'Effects of different levels of fish protein hydrolysate in the diet on the nonspecific immunity of Japanese sea bass, Lateolabrax japonicus (Cuvieret Valenciennes, 1828)', *Aquaculture Research*. John Wiley & Sons, Ltd (10.1111), 37(1), pp. 102–106. doi: 10.1111/j.1365-2109.2005.01392.x.

Liang, M. *et al.* (2006b) 'Effects of different levels of fish protein hydrolysate in the diet on the nonspecific immunity of Japanese sea bass, Lateolabrax japonicus (Cuvieret Valenciennes, 1828)', *Aquaculture Research*, 37(1), pp. 102–106. doi: 10.1111/j.1365-2109.2005.01392.x.

Lim, C. *et al.* (1997) 'Nutritive values of low and high fibre canola meals for shrimp (Penaeus vannamei)', *Aquaculture Nutrition*, 3(4), pp. 269–279. doi: 10.1046/j.1365-2095.1997.00048.x.

Lim, C. and Dominy, W. (1990) 'Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (Penaeus vannamei)', *Aquaculture*, 87(1), pp. 53–63. doi: 10.1016/0044-8486(90)90210-E.

Lim, K. C. *et al.* (2018) 'Astaxanthin as feed supplement in aquatic animals', *Reviews in Aquaculture*. John Wiley & Sons, Ltd (10.1111), 10(3), pp. 738–773. doi: 10.1111/raq.12200.

Liu, X. *et al.* (2012) 'Partial replacement of fish meal with peanut meal in practical diets for the Pacific white shrimp, Litopenaeus vannamei', *Aquaculture Research*, 43(5), pp. 745–755. doi: 10.1111/j.1365-2109.2011.02883.x.

Lopez-Cervantes, J., A, D. S.-M.-… of C. and 2006, U. (2006) 'High-performance liquid chromatography method for the simultaneous quantification of retinol, α-tocopherol, and cholesterol in shrimp waste hydrolysate', *Journal of Chromatography A*, 1105(1-2 SPEC. ISS.), pp. 135–139. doi: 10.1016/j.chroma.2005.08.010.

López-Cervantes, J., Sánchez-Machado, D. I. and Ríos-Vázquez, N. J. (2006) 'High-performance liquid chromatography method for the simultaneous quantification of retinol, α-tocopherol,

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

and cholesterol in shrimp waste hydrolysate', in *Journal of Chromatography A*. Elsevier, pp. 135–139. doi: 10.1016/j.chroma.2005.08.010.

Magnadóttir, B. (2006a) 'Innate immunity of fish (overview)', *Fish & Shellfish Immunology*. Academic Press, 20(2), pp. 137–151. doi: 10.1016/J.FSI.2004.09.006.

Magnadóttir, B. (2006b) 'Innate immunity of fish (overview)', in *Fish and Shellfish Immunology*. Academic Press, pp. 137–151. doi: 10.1016/j.fsi.2004.09.006.

Mai, K. *et al.* (2006) 'Effects of dietary squid viscera meal on growth and cadmium accumulation in tissues of Japanese seabass, Lateolabrax japonicus (Cuvier 1828)', *Aquaculture Research*, 37(11), pp. 1063–1069. doi: 10.1111/j.1365-2109.2006.01529.x.

Marchbank, T., Elia, G. and Playford, R. J. (2009) 'Intestinal protective effect of a commercial fish protein hydrolysate preparation', *Regulatory Peptides*, 155(1–3), pp. 105–109. doi: 10.1016/j.regpep.2009.02.003.

Mcgoogan, B. B. and Gatlin, D. M. (1997) 'Effects of replacing fish meal with soybean meal in diets for red drum Sciaenops ocellatus and potential for palatability enhancement', *Journal of the World Aquaculture Society*. World Aquaculture Society, 28(4), pp. 374–385. doi: 10.1111/j.1749-7345.1997.tb00284.x.

Médale, F. and Kaushik, S. (2009) 'Les sources protéiques dans les aliments pour les poissons d'élevage', *Cahiers Agricultures*. EDP Sciences, 18(2), pp. 103–111. doi: 10.1684/agr.2009.0279.

Meyers, S. P. (1986) 'Utilization of Shrimp Processing Wastes', *Infofish Marketing Dig*, 4(86), pp. 18–19. Available at: https://ci.nii.ac.jp/naid/10014713776/ (Accessed: 9 August 2019).

Millamena, O. M. (2002) 'Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper Epinephelus coioides', *Aquaculture*, 204(1–2), pp. 75–84. doi: 10.1016/S0044-8486(01)00629-9.

Nates, S. F. (2015) *Aquafeed Formulation*, *Aquafeed Formulation*. Elsevier Inc. doi: 10.1016/C2013-0-18878-2.

Naylor, R. L. *et al.* (2009) 'Feeding aquaculture in an era of finite resources', *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, pp. 15103–15110. doi: 10.1073/pnas.0905235106.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

‘New Method For Making Krill Meal’ (2013). Available at: https://patents.google.com/patent/US20140107072A1/en (Accessed: 9 August 2019).

Nguyen, H. T. M., Pérez-Gálvez, R. and Bergé, J. P. (2012) ‘Effect of diets containing tuna head hydrolysates on the survival and growth of shrimp Penaeus vannamei’, *Aquaculture*, 324–325, pp. 127–134. doi: 10.1016/j.aquaculture.2011.11.014.

NII, Y. *et al.* (2008) ‘Determination of Antihypertensive Peptides from an Izumi Shrimp Hydrolysate’, *Bioscience, Biotechnology, and Biochemistry*. Japan Society for Bioscience, Biotechnology, and Agrochemistry, 72(3), pp. 861–864. doi: 10.1271/bbb.70565.

Nunes, A. J. P., Sá, M. V. C. and Sabry-Neto, H. (2011) ‘Growth performance of the white shrimp, Litopenaeus vannamei, fed on practical diets with increasing levels of the Antarctic krill meal, Euphausia superba, reared in clear- versus green-water culture tanks’, *Aquaculture Nutrition*, 17(2). doi: 10.1111/j.1365-2095.2010.00791.x.

Nwanna, L. C. (2009) ‘Nutritional Value and Digestibility of Fermented Shrimp Head Waste Meal by African Catfish Clarias gariepinus’, *Pakistan Journal of Nutrition*, 2(6), pp. 339–345. doi: 10.3923/pjn.2003.339.345.

Olsen, R. E. *et al.* (2006) ‘The replacement of fish meal with Antarctic krill, Euphausia superba in diets for Atlantic salmon, Salmo salar’, *Aquaculture Nutrition*. John Wiley & Sons, Ltd (10.1111), 12(4), pp. 280–290. doi: 10.1111/j.1365-2095.2006.00400.x.

Ostaszewska, T. *et al.* (2010) ‘The effect of plant protein-based diet supplemented with dipeptide or free amino acids on digestive tract morphology and PepT1 and PepT2 expressions in common carp (Cyprinus carpio L.)’, *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 157(2), pp. 158–169. doi: 10.1016/j.cbpa.2010.06.162.

Paripatananont, T. *et al.* (2001) ‘Substitution of soy protein concentrate for fishmeal in diets of tiger shrimp *Penaeus monodon*’, *Aquaculture Research*, 32, pp. 369–374. doi: 10.1046/j.1355-557x.2001.00045.x.

Plascencia-Jatomea, M. *et al.* (2002a) ‘Feasibility of fishmeal replacement by shrimp head silage protein hydrolysate in Nile tilapia (Oreochromis niloticus L) diets’, *Journal of the Science of Food and Agriculture*. John Wiley & Sons, Ltd, 82(7), pp. 753–759. doi: 10.1002/jsfa.1092.

Plascencia-Jatomea, M. *et al.* (2002b) ‘Feasibility of fishmeal replacement by shrimp head silage protein hydrolysate in Nile tilapia (Oreochromis niloticus L) diets’, *Journal of the Science of*

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Food and Agriculture. John Wiley & Sons, Ltd, 82(7), pp. 753–759. doi: 10.1002/jsfa.1092.

Qian, Z. J., Je, J. Y. and Kim, S. K. (2007) 'Antihypertensive effect of angiotensin I converting enzyme-inhibitory peptide from hydrolysates of bigeye tuna dark muscle, Thunnus obesus', *Journal of Agricultural and Food Chemistry*, 55(21), pp. 8398–8403. doi: 10.1021/jf0710635.

Raghavan, S. and Kristinsson, H. G. (2009) 'ACE-inhibitory activity of tilapia protein hydrolysates', *Food Chemistry*, 117(4), pp. 582–588. doi: 10.1016/j.foodchem.2009.04.058.

Raghavan, S. and Kristinsson, H. G. (no date) 'Antioxidative Efficacy of Alkali-Treated Tilapia Protein Hydrolysates: A Comparative Study of Five Enzymes'. doi: 10.1021/jf0733160.

Raghavan, S., Kristinsson, H. G. and Leeuwenburgh, C. (2008) 'Radical Scavenging and Reducing Ability of Tilapia (*Oreochromis niloticus*) Protein Hydrolysates', *Journal of Agricultural and Food Chemistry*, 56(21), pp. 10359–10367. doi: 10.1021/jf8017194.

Richard, L. *et al.* (2010) 'Maintenance and growth requirements for nitrogen, lysine and methionine and their utilisation efficiencies in juvenile black tiger shrimp, Penaeus monodon, using a factorial approach', *British Journal of Nutrition*. Cambridge University Press, 103(7), pp. 984–995. doi: 10.1017/S0007114509992844.

Ringø, E. *et al.* (2012) 'Use of chitin and krill in aquaculture - the effect on gut microbiota and the immune system: A review', *Aquaculture Nutrition*. John Wiley & Sons, Ltd (10.1111), pp. 117–131. doi: 10.1111/j.1365-2095.2011.00919.x.

Romero, J. J. *et al.* (1994) 'Evaluation of methods to certify the "premium" quality of Chilean fish meals', *Aquaculture*, 124(1–4), pp. 351–358. doi: 10.1016/0044-8486(94)90408-1.

Saidi, S. *et al.* (2014) 'Production and fractionation of tuna by-product protein hydrolysate by ultrafiltration and nanofiltration: Impact on interesting peptides fractions and nutritional properties', *Food Research International*. Elsevier Ltd, 65(PC), pp. 453–461. doi: 10.1016/j.foodres.2014.04.026.

Shahidi, F. and Synowiecki, J. (1991) 'Isolation and Characterization of Nutrients and Value-Added Products from Snow Crab (Chinoecetes Opilio) and Shrimp (Pandalus Borealis) Processing Discards', *Journal of Agricultural and Food Chemistry*, 39(8), pp. 1527–1532. doi: 10.1021/jf00008a032.

SHEEHAN, D. *et al.* (2001) 'Structure, function and evolution of glutathione transferases:

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

implications for classification of non-mammalian members of an ancient enzyme superfamily', *Biochemical Journal*. Portland Press Ltd., 360(1), pp. 1–16. doi: 10.1042/bj3600001.

Shimizu, C. *et al.* (1990) 'Feeding stimulation in sea bream, Pagrus major, fed diets supplemented with Antarctic krill meals', *Aquaculture*. Elsevier, 89(1), pp. 43–53. doi: 10.1016/0044-8486(90)90232-C.

Siddik, M. A. B., Howieson, J. and Fotedar, R. (2019a) 'Beneficial effects of tuna hydrolysate in poultry by-product meal diets on growth, immune response, intestinal health and disease resistance to Vibrio harveyi in juvenile barramundi, Lates calcarifer', *Fish & Shellfish Immunology*. Academic Press, 89, pp. 61–70. doi: 10.1016/J.FSI.2019.03.042.

Siddik, M. A. B., Howieson, J. and Fotedar, R. (2019b) 'Beneficial effects of tuna hydrolysate in poultry by-product meal diets on growth, immune response, intestinal health and disease resistance to Vibrio harveyi in juvenile barramundi, Lates calcarifer', *Fish and Shellfish Immunology*. Academic Press, 89, pp. 61–70. doi: 10.1016/j.fsi.2019.03.042.

Sila, A. *et al.* (2014) 'Antibacterial peptides from barbel muscle protein hydrolysates: Activity against some pathogenic bacteria', *LWT - Food Science and Technology*, 55(1), pp. 183–188. doi: 10.1016/j.lwt.2013.07.021.

Silva-Carrillo, Y. *et al.* (2012) 'The effect of substituting fish meal with soybean meal on growth, feed efficiency, body composition and blood chemistry in juvenile spotted rose snapper Lutjanus guttatus (Steindachner, 1869)', *Aquaculture*. Elsevier, 364–365, pp. 180–185. doi: 10.1016/j.aquaculture.2012.08.007.

Smith, D. M. *et al.* (2005) 'The efficacy of ingredients included in shrimp feeds to stimulate intake', *Aquaculture Nutrition*, 11(4), pp. 263–272. doi: 10.1111/j.1365-2095.2005.00349.x.

SMITH, D. M. *et al.* (2005) 'The efficacy of ingredients included in shrimp feeds to stimulate intake', *Aquaculture Nutrition*, 11(4), pp. 263–272. doi: 10.1111/j.1365-2095.2005.00349.x.

Smith, V. J. *et al.* (2000) 'Antibacterial proteins in rainbow trout, Oncorhynchus mykiss', *Fish & Shellfish Immunology*, 10, pp. 243–260. doi: 10.1006/fsim.1999·0254.

Soares, J. *et al.* (no date) 'Chemical composition of Atlantic and Gulf menhaden fish solubles', *MILLER PUBL CO 2501 WAYZATA ….*

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

Steffens, W. (1994) 'Replacing fish meal with poultry by-product meal in diets for rainbow trout, Oncorhynchus mykiss', *Aquaculture*, 124(1–4), pp. 27–34. doi: 10.1016/0044-8486(94)90351-4.

Suárez, J. A. *et al.* (2009) 'Substitution of fish meal with plant protein sources and energy budget for white shrimp Litopenaeus vannamei (Boone, 1931)', *Aquaculture*, 289(1–2), pp. 118–123. doi: 10.1016/j.aquaculture.2009.01.001.

Suresh, A. V., Kumaraguru vasagam, K. P. and Nates, S. (2011) 'Attractability and palatability of protein ingredients of aquatic and terrestrial animal origin, and their practical value for blue shrimp, Litopenaeus stylirostris fed diets formulated with high levels of poultry byproduct meal', *Aquaculture*, 319(1–2), pp. 132–140. doi: 10.1016/j.aquaculture.2011.06.039.

Tacon, A. G. J. and Barg, U. C. (1998) 'Major Challenges to Feed Development for Marine and Diadromous Finfish and Crustacean Species', in *Tropical Mariculture*. Elsevier, pp. 171–207. doi: 10.1016/b978-012210845-7/50006-4.

Tacon, A. G. J. and Metian, M. (2009) 'Fishing for feed or fishing for food: Increasing global competition for small pelagic forage fish', *Ambio*. SpringerRoyal Swedish Academy of Sciences, 38(6), pp. 294–302. doi: 10.1579/08-A-574.1.

Takagi, S. *et al.* (2001) 'Effect of lysine and methionine supplementation to a soy protein concentrate diet for red sea bream Pagrus major', *Fisheries Science*. John Wiley & Sons, Ltd (10.1111), 67(6), pp. 1088–1096. doi: 10.1046/j.1444-2906.2001.00365.x.

Takii, K. *et al.* (1997) 'Influence of Feeding Rates on Digestion and Energy Flow in Tiger Puffer and Red Sea Bream', *Fisheries science*. The Japanese Society of Fisheries Science, 63(3), pp. 355–360. doi: 10.2331/fishsci.63.355.

Tang, H. G. *et al.* (2008) 'Effects of fish protein hydrolysate on growth performance and humoral immune response in large yellow croaker (Pseudosciaena crocea R.)', *Journal of Zhejiang University: Science B*, 9(9), pp. 684–690. doi: 10.1631/jzus.B0820088.

Teshima, S.-I. *et al.* (2004) 'Effects of protein and lipid sources on the growth and survival of red sea bream Pagrus major and Japanese flounder Paralichthys olivaceus receiving micro-bound diets during larval and early juvenile stage', *Aquaculture Nutrition*. John Wiley & Sons, Ltd (10.1111), 10(4), pp. 279–287. doi: 10.1111/j.1365-2095.2004.00303.x.

TESSER, M. B. *et al.* (2005) 'Free- and peptide-based dietary arginine supplementation for the South

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

American fish pacu (Piaractus mesopotamicus)', *Aquaculture Nutrition*, 11(6), pp. 443–453. doi: 10.1111/j.1365-2095.2005.00373.x.

The State of World Fisheries and Aquaculture 2018 (2018). UN (The State of World Fisheries and Aquaculture). doi: 10.18356/8d6ea4b6-en.

Tibbetts, S. M., Milley, J. E. and Lall, S. P. (2006) 'Apparent protein and energy digestibility of common and alternative feed ingredients by Atlantic cod, Gadus morhua (Linnaeus, 1758)', *Aquaculture*. Elsevier, 261(4), pp. 1314–1327. doi: 10.1016/J.AQUACULTURE.2006.08.052.

Twibell, R. G. and Wilson, R. P. (2002) 'Nutrient requirements and feeding of finfish for aquaculture', *Aquaculture*. CABI, 214(1–4), pp. 419–420. doi: 10.1016/s0044-8486(02)00345-9.

Vergara, J. M. *et al.* (1999) 'Growth, feed utilization and body lipid content of gilthead seabream (Sparus aurata) fed increasing lipid levels and fish meals of different quality', *Aquaculture*, 179(1–4), pp. 35–44. doi: 10.1016/S0044-8486(99)00150-7.

Wang, Y. *et al.* (2006) 'Replacement of fish meal by rendered animal protein ingredients in feeds for cuneate drum (Nibea miichthioides)', *Aquaculture*, 252(2–4), pp. 476–483. doi: 10.1016/j.aquaculture.2005.07.018.

Webster, C. D., Goodgame-Tiu, L. S. and Tidwell, J. H. (1995) 'Total replacement of fish meal by soy bean meal, with various percentages of supplemental L-methionine, in diets for blue catfish, Ictalurus furcatus (Lesueur)', *Aquaculture Research*, 26(5), pp. 299–306. doi: 10.1111/j.1365-2109.1995.tb00917.x.

Worldwide Fish Meal Production Outlook and the Use of Alternative Protein Meals for Aquaculture | W. Hardy | Avances en Nutrición Acuicola (no date). Available at: http://nutricionacuicola.uanl.mx/index.php/acu/article/view/179 (Accessed: 29 November 2019).

Wu, G., Physiology, D. S.-P. and 1995, undefined (no date) 'Isolation and characterization of a potato catalase cDNA (GenBank U27082)'.

Wyban, J., Walsh, W. A. and Godin, D. M. (1995) 'Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (Penaeus vannamei)', *Aquaculture*, 138(1–4), pp. 267–279. doi: 10.1016/0044-8486(95)00032-1.

Xie, F. *et al.* (2012) 'Dietary lysine requirement of juvenile Pacific white shrimp, Litopenaeus

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY

vannamei', *Aquaculture*, 358–359, pp. 116–121. doi: 10.1016/j.aquaculture.2012.06.027.

Xie, S. *et al.* (2019) 'Dietary replacement of fish-meal impaired protein synthesis and immune response of juvenile Pacific white shrimp, Litopenaeus vannamei at low salinity', *Comparative Biochemistry and Physiology Part - B: Biochemistry and Molecular Biology*. Elsevier Inc., 228, pp. 26–33. doi: 10.1016/j.cbpb.2018.11.002.

Yamamoto, T. *et al.* (1998) 'Apparent and True Availabilities of Amino Acids from Several Protein Sources for Fingerling Rainbow Trout, Common Carp, and Red Sea Bream', *Fisheries science*. The Japanese Society of Fisheries Science, 64(3), pp. 448–458. doi: 10.2331/fishsci.64.448.

Yan, J. *et al.* (2018) 'Effect of Dietary Antarctic Krill Meal on Growth Performance, Muscle Proximate Composition, and Antioxidative Capacity of Juvenile Spotted Halibut, Verasper variegatus', *Journal of the World Aquaculture Society*. John Wiley & Sons, Ltd (10.1111), 49(4), pp. 761–769. doi: 10.1111/jwas.12455.

Yang, R. *et al.* (2009) 'Immunomodulatory effects of marine oligopeptide preparation from Chum Salmon (Oncorhynchus keta) in mice', *Food Chemistry*, 113(2), pp. 464–470. doi: 10.1016/j.foodchem.2008.07.086.

Yang, Y. *et al.* (2004) 'Effect of replacement of dietary fish meal by meat and bone meal and poultry by-product meal on growth and feed utilization of gibel carp, Carassius auratus gibelio', *Aquaculture Nutrition*, 10(5), pp. 289–294. doi: 10.1111/j.1365-2095.2004.00301.x.

Yoshitomi, B. *et al.* (2006) 'Evaluation of krill (Euphausia superba) meal as a partial replacement for fish meal in rainbow trout (Oncorhynchus mykiss) diets', *Aquaculture*. Elsevier, 261(1), pp. 440–446. doi: 10.1016/j.aquaculture.2006.06.036.

제주대학교 중앙도서관
JEJU NATIONAL UNIVERSITY LIBRARY