Department of Environment and Agriculture

Supplementation of Organic Selenium to Improve Inclusion Level of Plant-derived Proteins in the Diets of Two Commercially Important Marine Carnivorous Finfish Species

Ilham

This thesis is presented for the Degree of
Doctor of Philosophy
of
Curtin University

May 2017

Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council Australian code for the care and use of animals for scientific purposes 8th edition (2013). The proposed research study received animal ethics approval from the Curtin University Animal Ethics Committee, Approval Number #AEC_2013_07.

Signature:

Date: 01/05/2017

ABSTRACT

Fish feeds account for more than half the production cost in most commercial aquaculture operation today, with fishmeal (FM) being the traditional protein source in aquaculture feeds. Increased demand for aquaculture products has skyrocketed FM prices over the last few decades, thereby mounting the pressure on the wild fish stocks. Therefore, the search for lower cost, sustainable protein ingredients continues to gain momentum. Plant protein (PP) ingredients such as soybean meal (SBM) and lupin meal (LM) have excellent nutritional properties, making them exceptional candidates as FM substitutes. However, one identified drawback with the use of plant-based diets is the presence of certain anti-nutritional factors (ANF), which can reduce mineral bioavailability for fish and therefore induce depressed growth.

To enable a continued improvement of feed formulation, and plant-based ingredients' use in fish feeds, a series of experiments were conducted to evaluate whether dietary supplementation of selenium (Se) affects the growth, enzymatic antioxidant capacity, and health status of carnivorous marine finfish species. The first objective of this research was to investigate the effects of dietary organic Se (OS, Sel-Plex®) on growth, glutathione peroxidase activity, and biochemical status of juvenile yellowtail kingfish (*Seriola lalandi*), when reared at different temperatures and when exposed to dietary PP sources. Another objective was to evaluate whether OS supplementation and or plant ingredients fermentation influenced growth, feed utilisation, muscle Se content, enzymatic antioxidant activity of glutathione peroxidase (GPx), histopathology, and blood biochemistry of barramundi fed diets containing PP products.

In yellowtail kingfish, it was demonstrated that there is a synergistic effect between Se level and temperature, which was reflected by a superior increase in final weight (FW) and specific growth rate (SGR) in fish supplemented with 2.0 mg Se kg^{-1}at 21ºC than at 26ºC ($P < 0.001$). Enzymatic GPx activity levels showed that OS supplementation could enhance the physiological response during stress periods. In another experiment, with OS supplementation, 25% FM protein could be replaced by SBM in the diets of yellowtail kingfish. Conversely, syndromes including retarded growth, lower GPx activity, and alterations in tissue structure were observed in fish

fed high SBM (75%) diets. The feeding trials with yellowtail kingfish have demonstrated that OS supplementation may be required to avoid deficiency syndromes, to maintain maximum growth, and to enhance a functional immune system in yellowtail kingfish. Meanwhile, for barramundi (*Lates calcarifer*), dietary requirement of 3.5–4.5 mg Se kg^{-1} is necessary when high level of PP ingredients is included in the diets. However, the findings from both SBM and LM feeding trials showed that GPx is a beneficial marker of Se deficiency in barramundi. Although the kidney, spleen, and intestine were histologically normal, severe necrosis of muscle fibres was found in the liver of barramundi fed diets either deficient or excessive in Se. Furthermore, phytate may interfere with trace mineral absorption and utilisation in fish; however, fermentation with *Saccharomyces cerevisiae* was able to degrade antinutritional phytate contained in PP-based diets. In line with improvement of nutritional value and functional properties of SBM and LM proteins, fermented PP products and OS supplementation could also enhance FW, SGR, WG, and GPx activity of barramundi. Moreover, the proposed feed formulation strategies play an important role in maintaining an appropriate biochemical status of the fish.

The findings of this research may contribute towards the production of an economically friendly and ecologically sustainable diets in aquaculture, especially for carnivorous marine finfish species. What is more, the outcome of this study may be of interest to grain growers, aquaculturists, retailers, consumers, and researchers, all of whom are concerned with the use of plant-based products in the diets of cultured fish.

ACKNOWLEDGEMENTS

First, I wish to express my special thanks to my PhD Supervisor, Prof. Ravi Fotedar for his valuable guidance, as well as all the affection, encouragement and support during the whole stages of my PhD program. Most importantly, I am indebted to him for being a friend and for coaching me as a scientist, lessons which show me how science can contribute to the benefits of the world.

Sincere appreciation goes to the Australia Awards Scholarship Council for fully sponsoring my PhD study. I am intensely indebted to the staff members of Curtin University's International Sponsored Students Unit, Julie Craig, Christine Kerin, Kristen Soon, and Hoa Pham for all their valuable support and assistances on both personal and academic affairs, which are fondly remembered.

I would like to thank my colleagues at Curtin Aquatic Research Laboratory, Sulaeman, Irfan Ambas, Rudy Nugroho, Ardiansyah, Muhammad Abu Bakar Siddik, Dinh Quang Huy, Mai Van Ha, and Antony Cole for all their support and friendship during my Curtin life. I thank Simon Longbottom, Rowan Kleindeist, Muhammad Yasier, Munilkumar Sukham and Himawan Achmad for their technical help during experimental and sampling period. I also would like to thank Fran Stephens and Mai Cam Nguyen from the Department of Agriculture and Food Western Australia for their laboratory assistances.

I take this opportunity to thank staffs from Aquaculture TAFE, Challenger Institute of Technology, Fremantle for providing yellowtail kingfish and barramundi juveniles for all of my experiments. Also, I would like to thank Warren Potts, Specialty Feeds, and Alltech Company based in Australia for their feed ingredients supply used for my fish nutrition research.

This PhD journey would not have been possible without support from my home institution, Jakarta Fisheries University, Ministry of Marine Affairs. My special thanks go to Aef Permadi, I Nyoman Suyasa, Moch. Nurhudah, Effi A. Thaib, Sinung Rahardjo, and Irfansyah for their continuous support during my PhD study. I offer my deep appreciation to Fitriska Hapsari and Meuthia Aula Jabbar for their friendship, assistance, valuable information, and technical support at all times.

Back to Australia, I was very fortunate to have met many good friends who have made this journey an unforgettable one. I am grateful to all of you: Tubagus Solihuddin, Bambang Widyo Prastowo, Achmad Room Fitriyanto, Akhdian Reppawali, Budi Cahyono, Moch. Khobir, Joni Adiansyah, Ahmad Jauhari, Isaaq Syahputra, and Thya.

Finally, I would like to express my heartiest thanks to my family, my wife Idamayanti, my daughters Nadeen and Fiona whose proximity, love and affection instil me joy and relaxation though they sacrificed a lot of my company. Especially, I would like to offer my special thanks and deep appreciation to my beloved wife for her efforts and cheerful encouragement during the period of this study. I have also highly indebted to my mother, late father, and my sisters for their important support.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

AA	Amino acid
ABARES	Australian Bureau of Agricultural and Resource Economics and Sciences
ACAAR	Australian Centre for Applied Aquaculture Research
ADC	Apparent digestibility coefficient
ALT	Alanine aminotransferase
ANF	Anti-nutritional factor
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BW	Body weight
Ca	Calcium
CARL	Curtin Aquatic Research Laboratory
CAT	Catalase
CK	Creatinine kinase
Co	Cobalt
Cr_2O_3	Chromic oxide
Cu	Copper
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
FAO	Food and Agriculture Organisation
FCR	Feed conversion ratio
Fe	Ferrum (iron)
FI	Feed intake
FO	Fish oil
FM	Fishmeal
FW	Final weight
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSSG	Oxidised glutathione
HB	Haemoglobin
H_2O_2	Hydrogen peroxide
ICP-AES	Inductively coupled plasma atomic emission spectrometry

IW	Initial weight
LM	Lupin meal
Mg	Magnesium
Mn	Manganese
NA	Not Analysed
NADPH	Nicotinamide adenine dinucleotide phosphate
Ni	Nickel
NRC	National Research Council
OECD	Organisation for Economic Cooperation and Development
OS	Organic selenium
PP	Plant protein
PUFA	Poly-unsaturated fatty acid
RBC	Red blood cell
RFI	Relative feed intake
RMR	Routine metabolic rate
ROS	Reactive oxygen species
SBM	Soybean meal
Se	Selenium
SE	Standard error
Se-Cys	Selenocysteine
Se-Met	Seleno-methionine
Se-Se-Se	Triselenium linkage
Se-yeast	Seleno-yeast
SGR	Specific growth rate
SOD	Superoxide dismutase
SPC	Soy protein concentrate
SPSS	Statistical package for social science
S-S	Disulfide
S-Se-S	Selenotrisulfide linkage
UK	United Kingdom
US-EPA	United States Environmental Protection Agency
WA	Western Australia
WG	Weight gain
Zn	Zinc

LIST OF ANIMALS

Abalone (*Haliotis discus hannai Ino*)

African catfish (*Clarias gariepinus*)

Atlantic cod (*Gadus morhua*)

Atlantic salmon (*Salmo salar*)

Barramundi (*Lates calcarifer*)

Beluga (*Huso huso*)

Black sea bream (*Acanthopagrus schlegelii*)

Bluegill sunfish (*Lepmis macrochirus*)

Broiler chicken (*Gallus gallus*)

Caspian brown trout (*Salmo trutta caspius*)

Channel catfish (*Ictalurus punctatus*)

Chinese sucker (*Myxocyprinus asiaticus*)

Chinook salmon (*Oncorhynchus tshawytscha*)

Chum salmon (*Oncorhynchus keta*)

Cobia (*Rachycentron canadum*)

Cod (*Godus morhua*)

Coho salmon (*Oncorhynchus kisutch*)

Common carp (*Cyprinus carpio*)

Crayfish (*Procambarus clarkii*)

Crucian carp (*Carassius auratus gibelio*)

Cuneate drum (Nibea miichthioides)

Cutthroat trout (*Oncorhynchus clarkia*)

European sea bass (*Dicentrarchus labrax)*

Freshwater prawn (*Macrobachium rosenbergii*)

Gibel carp (*Carassius auratus gibelio*)

Gilthead sea bream (*Sparus aurata*)

Great sturgeon (*Huso huso*)

Green sturgeon (*Acipenser medirostris*)

Grouper (*Ephinephelus malabaricus*)

Hybrid striped bass (*Morone chrysops* × *M. saxatilis*)

Hybrid tilapia (*Oreochromis niloticus* × *O. aureus*)

Indian white shrimp (*Fenneropenaeus indicus*)

Japanese flounder (*Paralichthys olivaceus*)

Japanese yellowtail (*Seriola quinqueradiata*)

Korean rockfish (*Sebastes schlegeli*)

Largemouth bass (*Micropterus salmoide*)

Loach (*Paramisgurnus dabryanus*)

Marron (*Cherax cainii*)

Mediterranean yellowtail (*Seriola dumerili*)

Nile tilapia (*Oreochromis niloticus*)

Planktonic crustacean (*Daphnia magna*)

Pompano (*Trachinotus ovatus*)

Rainbow trout (*Oncorhynchus mykiss*)

Red hybrid tilapia (*Oreochromis sp.*)

Red sea bream (*Pagrus major*)

Rohu (*Labeo rohita*)

Sea bass (*Dicentrarchus labrax*)

Sharpsnout seabream (*Diplodus puntazzo*)

Spotted rose snapper (*Lutjanus guttatus*)

Striped bass (*Morone saxatilis*)

Tilapia (*Oreochromis niloticus*)

Totoaba (*Totoaba macdonaldi*)

Turbot (*Scophthalmus maximus L.*)

White sturgeon (*Acipenser transmontanus*)

Whiteleg shrimp (*Litopenaeus vannamei*)

Yellowtail kingfish (*Seriola lalandi*)

Zebrafish (*Danio rerio*)

PREAMBLE

Mineral nutrition remains the least eminent subject of fish nutrition studies. Available data and information on the requirements of trace mineral are quite limited in comparison to data for other nutrients. It has also been recognised that the raw materials currently used in aquaculture feed contain varying amount of mineral contents. While most of the research to date has focused on the significance of dietary trace mineral in fish fed with fishmeal, almost no information is present on the effect of plant protein practical diets on the dietary trace element requirement to fish. The current study was accomplished to precisely comprehend the growth and health implications of selenium (Se) supplementation in the diets of two commercially important carnivorous marine finfish species, these being yellowtail kingfish (*Seriola lalandi*) and barramundi (*Lates calcarifer*), fed plant-based protein sources. The efficacy of organic Se (OS; Sel-Plex®) supplementation on the performance of these two aquaculture species was assessed by investigating their growth performances, nutrient utilisation, enzymatic antioxidant activity, tissue Se content, histopathology of various internal organs, and physiological and biochemical status.

In general, this dissertation consists of nine chapters. The 1st chapter covers an introduction that highlight the importance of Se nutrition in aquaculture species, particularly when they are fed diets containing plant-based proteins. The use of plant-derived ingredients as the major dietary protein source in carnivorous marine finfish diets is discussed. Research aim and objectives are also included in this introductory chapter.

The 2nd chapter provides an overview of existing literatures on three relevant aspects. Firstly, a general overview of yellowtail kingfish and barramundi aquaculture, with the major focus on their nutrition; secondly, information regarding the use of plant-derived protein sources such as soybean meal and lupin meal as alternatives to fishmeal; and thirdly, the role of Se as feed supplement in aquaculture. At the end, this chapter provides a summary that ties together all of the information related to the foresaid aspects.

Chapter 3 to Chapter 8 examine the efficacy of dietary OS supplementation on yellowtail kingfish and barramundi growth, physiological and histological performances. Parameters investigated include growth indices, nutrient utilisation, antioxidant activity, muscle Se content, histopathology, as well as blood chemistry. The main text of all these chapters is written in "manuscript" style appropriate for publication to a scientific journal. Therefore, some of the background information appeared in "introduction" and "materials and methods" sections of these chapters may be repetitive in nature, despite careful re-writing to avoid unnecessary duplication. These chapters have been either published (Chapter 3 to Chapter 7) or accepted for publication (Chapter 8) in peer-review journals.

Chapter 3 describes an experiment with the objective of examining the effects of dietary OS in yellowtail kingfish. The experiment employed a factorial approach to evaluating growth, antioxidant activity, muscle Se and histopathology of yellowtail kingfish fed diets supplemented with three OS levels and reared at two temperatures. The content of this chapter has been published in "Animal Feed Science and Technology". The effects of dietary soybean meal (SBM) supplemented with OS on growth, digestibility, muscle Se level, and histological alterations are described in Chapter 4, as published in "International Journal of Food and Nutritional Science".

Chapter 5 portrays the performance of juvenile barramundi when fed diets containing OS and SBM. Two experiments were conducted to determine the implications of dietary OS on the growth, feed utilisation, antioxidant activity, tissue Se accumulation, and histological changes of the fish fed low and high SBM diets. This chapter has been published in "Biological Trace Element Research". The potential application of another plant-derived ingredient, lupin meal (LM), in diets of barramundi is described in Chapter 6. A version of this chapter has been published in "Aquaculture".

Both Chapter 7 and Chapter 8 elaborates the potential benefits of using fermented plant-based products simultaneously supplemented with OS in enhancing growth and health status of barramundi. Whilst Chapter 7 deals with an experiment using fermented SBM in barramundi diets (published in "Fish Physiology and Biochemistry), Chapter 8 describes an experiment that focused on the utilisation of fermented LM in diets of barramundi (Published in "Aquaculture Research").

The 9th Chapter attempts to present all the findings together by discussing all-embracing effects of dietary OS and PP products on the growth and health performances of these two carnivorous marine finfish species. Thus, extraction of the data using statistical analysis tools is needed to highlight the key suppositions of the research. Some conclusions and recommendations are included in this chapter.

CHAPTER 1: Introduction

1.1. Background

Aquaculture, the farming of aquatic organisms, has been considered as the fastest emerging form of animal food production in the world. In 2012, aquaculture supplied approximately half of fish destined for human consumption (FAO, 2014). The key drivers for this outstanding performance of the industry include market demand, environments, infrastructure, technical capability, investment, human resources and institutional system (Bostock et al., 2010). However, concerns about sustainability have surrounded the development of marine aquaculture sector throughout much of the past decades. The feeds cost and disease outbreaks are two of the major challenges to the achievement of sustainable marine aquaculture.

Although many advances have been documented in substituting levels of fishmeal (FM) in aquaculture feeds with alternative protein sources (Hardy, 2010), it is apparent that FM remains the major component of the diets of popular marine carnivorous species (Hasan et al., 2011), particularly those considered 'new' in aquaculture such as yellowtail kingfish. With the continued growth in the farming of carnivorous species, the demand for FM is projected to keep rising, thus upholding pressures on fishmeal prices. It has also been reported that carnivorous marine finfish consume substantially more FM protein than they yield (Allsopp et al., 2008 ; Huntington & Hasan, 2009). For example, current fish in fish out (FIFO) ratio for yellowtail kingfish is 1,9:1(Stone, 2012). Hence, from marine finfish aquaculture's perspective, moving towards more sustainable feeds from plant origins is seen as a fundamental approach to the issue. This effort necessitates more researches in the search for and, much importantly, formulation of eco-friendly, nutritionally balanced, and lower-cost feeds for sustainable development of aquaculture.

A variety of plant protein (PP) sources has been investigated as possible FM replacements for farmed marine carnivorous species, with soybean meal (SBM) most widely utilised (Swick, 2002). SBM is considered a safe, reliable and cost-effective protein source for marine finfish diets. The reasons for this are the security of supply, reasonable price, high digestibility and balanced amino acid (AA) composition (Gatlin et al., 2007 ; Watanabe, 2002). Successful replacement of FM with SBM has

been reported for a range of marine finfish species including spotted rode snapper *Lutjanus guttatus* (Silva-Carrillo et al., 2012), Japanese flounder *Paralichthys olivaceus* (Kikuchi, 1999), Korean rockfish *Sebastes schlegeli* (Lim et al., 2004), Atlantic cod *Gadus morhua* (Refstie et al., 2006a), sharpsnout seabream *Diplodus puntazzo* (Hernández et al., 2007) and cobia *Rachycentron canadum* (Chou et al., 2004). Substituting SBM for FM to provide dietary protein at low incorporation levels appeared to be fairly undemanding; however, high or complete replacement of fishmeal with SBM is problematic (Hardy, 2010). This has been closely associated with some unfavourable properties of SBM such as amino acid (AA) imbalances, mineral deficiencies, high carbohydrate content, poor palatability and the existence of anti-nutritional factors (ANF). The alteration from FM to plant-derived protein sources such as SBM might infer growth, metabolism and health status for the cultured fish. Therefore, challenges to retain excellent growth rates and feed efficiency values at higher or full incorporation levels of SBM should be resolved.

Methods of processing SBM have been developed to improve the nutritional potential of SBM through heating, alcohol extraction, bioprocessing or enzymatic treatment. Soybean products which are derived from SBM and have been used in marine finfish nutrition studies are, for example, solvent extracted SBM, full-fat SBM, dehulled SBM, fermented SBM, enzyme-treated SBM and soy protein concentrates (SPC) (Barrows et al., 2007 ; Bowyer et al., 2013a ; Bowyer et al., 2013b ; Drew et al., 2007 ; Kokou et al., 2012 ; Tibaldi et al., 2006). Yet, with the existing technological advancement in processing SBM, no method can remove all the ANF while conserving the protein content of SBM.

Commonly used plant-derived feed ingredients such as SBM contain 10–15 g kg^{-1} anti-nutritional phytate (Francis et al., 2001). Phytate chelates with di- and trivalent mineral cations, namely, Cu, Zn, Ni, Co, Mn, Fe, Se, Mg, and Ca (Connelly, 2011), thereby forming insoluble complexes in the upper gastrointestinal tract, where maximum mineral absorption typically occurs (Kumar et al., 2012). Although it has been acknowledged that incorporation of plant-derived protein sources into aquafeeds reduces the availability of mineral for marine finfish species, information on selenium (Se) composition of fish fed SBM diets do not exist.

Of the minerals required by the fish, Se is an essential trace element needed for normal growth and physiological function (Hamilton, 2004). Se occurs in two forms, inorganic Se and organic Se. However, in terms of bioavailability and effects on fish health, organic Se has been found to be more readily absorbed and more effective (Le & Fotedar, 2014a ; Lorentzen et al., 1994 ; Wang & Lovell, 1997 ; Wang et al., 2007). The use of organic Se as a dietary supplement has successfully enhanced the growth and survival performance of a variety of aquatic species, including African catfish *Clarias gariepinus* (Abdel-Tawwab et al., 2007), Coho salmon *Oncorhynchus kisutch* (Felton et al., 1996), crucian carp *Carassius auratus gibelio* (Wang et al., 2007), grouper *Ephinephelus malabaricus* (Lin & Shiau, 2005) and marron *Cherax cainii* (Nugroho & Fotedar, 2013). The importance of Se in aquatic animal nutrition is significantly growing accompanied by the intensification of the aquaculture industry.

Furthermore, Se has been found to be an integral component of glutathione peroxidase (GPx) (Rotruck et al., 1973), which is a very potent antioxidant in the protection mechanism against harmful effects due to oxidative cellular injury (Sweetman et al., 2010). GPx protects cells and membranes from oxidative damage by destroying hydrogen peroxide and hydroperoxides employing reducing equivalents from glutathione (Watanabe et al., 1997). The activity of GPx in the liver or in plasma is generally used as an indicator of the selenium status in the organism (Pedrero & Madrid, 2009). Decreased GPx activity caused by Se deficiency has been observed in rainbow trout, Atlantic salmon, channel catfish and tilapia (Atencio et al., 2009 ; Bell et al., 1987 ; Hilton et al., 1980 ; Wise et al., 1993). The antioxidant properties of GPx may be required by fish, particularly salmonids, at a relatively high level to protect their increased levels of poly-unsaturated fatty acids (PUFAs), which are susceptible to lipid peroxidation (Webster & Lim, 2002).

Dietary Se is also needed for the normal functioning of many facets of the immune system (Arthur et al., 2003 ; Kiremidjian-Schumacher & Stotzky, 1987). This is of particular importance for intensive marine aquaculture, in which stress-induced diseases have resulted in considerable losses. The established beneficial properties of Se supplement in a range of fish species promote its application for disease management strategies in sustainable aquaculture practices. Nevertheless, the

prevention of disease (proactive approach), from the economic and environmental point of view, is much more advantageous than the treatment of disease (reactive approach).

Back to the peculiarity of Se, despite being a well-known antioxidant with significant effects on the immune system, the threshold between toxicity and deficiency is very narrow (Thiry et al., 2012). Therefore, it is extremely necessary to ensure that both Se deficiency and Se excess levels are balanced in the diet for cultured fish. While Se deficiency leads to growth depression, low survival, nutritional muscular dystrophy, and reduced enzymatic activity of GPx (Bell et al., 1987 ; Bell et al., 1986 ; Gatlin et al., 1986 ; Poston et al., 1976b), Se excess causes reduced growth, increased mortality, abnormal erythrocytes, and liver and kidney damage (Coughlan & Velte, 1989 ; Hilton et al., 1980 ; Ogle & Knight, 1989 ; Vidal et al., 2005). The Se requirement of fish varies with the form of Se ingested, dietary PUFA and vitamin E, and waterborne Se concentration (NRC, 1993).

Recently, researchers at the Curtin Aquatic Research Laboratory (CARL), Curtin University, have initially investigated the Se nutrition in yellowtail kingfish *Seriola lalandi* fed FM diets. It was recommended that the level of dietary Se (Sel-Plex®) for the optimum growth of the species was 5.56 mg kg^{-1} (Le & Fotedar, 2013a), with seleno-methionine (Se-Met) and seleno-yeast (Se-Yeast) were the most bioavailable sources of Se (Le & Fotedar, 2014a). Furthermore, dietary Se intake enhanced GPx antioxidant activity during normal culture conditions and during the stress-challenge period (Le & Fotedar, 2014b ; Le et al., 2014b). Overall, although commercial FM diets contain Se at required levels, Se supplementation appears to be necessary to maintain growth, survival and health performance of the farmed species. However, one of the challenges to the development of carnivorous marine finfish aquaculture in Australia includes the reduction of FM use in aquafeeds through the provision of environmentally and economically sustainable diets that boost growth and survival. To this extent, research addressing efforts to replace FM with increased levels of PP ingredients for carnivorous marine finfish diets is gaining momentum.

1.2. Aims

The study pursued to investigate whether the inclusion level of FM in a commercial diet for carnivorous marine finfish (i.e. yellowtail kingfish and barramundi) can be improved with plant-derived protein products when these diets are supplemented with the organic Se. It is expected that knowledge and information obtained from this study would be of great importance in promoting sustainable marine finfish aquaculture practices.

1.3. Objectives

To achieve the above aims, the following specific objectives were established:

i. To evaluate the effect of organic Se supplementation on the growth performance, physiological responses and muscle histology of juvenile yellowtail kingfish fed fishmeal diets at two temperatures.
ii. To determine whether Se supplementation affects growth, physiological responses and muscle histopathology of yellowtail kingfish fed high amounts of SBM diets.
iii. To investigate the growth and health performance of barramundi fed diets containing high levels of SBM simultaneously supplemented with organic Se.
iv. To determine the optimum dietary supplementation of Se for barramundi when fed diets containing high levels of LM.
v. To examine the effects of refinement of PP ingredients (i.e. SBM and LM) through fermentation on fish growth and health performance.

CHAPTER 2: Literature Review

2.1. Carnivorous Marine Finfish Aquaculture

Today, more than half of fish production intended for human consumption are supplied by aquaculture (FAO, 2016). Increased demand for food fish accelerates the growth of the industry at an average level of 8–10% every year (Teves & Ragaza, 2016). Finfish farming is the most highly industrialised aquaculture due to its productivity growth and technological advancement. By 2014, an amount of 362 finfish species is cultivated around the world (FAO, 2016).

In Australia, the total production volume of the aquaculture sector dropped by 2% in 2013, which was caused by a reduction in the finfish and crustaceans production by around 4% and 7%, respectively (Australian Bureau of Agricultural and Resource Economics and Sciences, 2014). Marine finfish, including salmonids, tuna, yellowtail kingfish, and barramundi, are the largest contributor to Australian aquaculture production (ABARES, 2014). It has been established that a range of hindrances to reaching anticipated growth of this industry does exist. These involve the need for enabling investment, an increase in resource use efficiency, an expansion in trade, and enhancing environmentally friendly aquaculture (FAO, 2016). However, marine finfish aquaculture industry should demonstrate that it is running within the notions of environmentally sustainable development. One of the global concerns is that increased aquaculture production will increase reliance on fishmeal (FM) and fish oil (FO), and consequently place hefty risk for the industry (Tacon & Metian, 2008). In fact, most traded farmed marine fish species are carnivorous and require 300-500 g kg^{-1} dietary FM (Tacon & Metian, 2008).

2.1.1. Yellowtail kingfish

Yellowtail kingfish (*Seriola lalandi*; Carangidae) is a pelagic marine carnivore and distributed globally around temperate and subtropical water environments (Booth et al., 2013 ; Fielder & Heasman, 2011 ; Poortenaar et al., 2001). While the culture of yellowtail kingfish (*Seriola lalandi*; Carrangidae) in Japan has occurred for many years, yellowtail kingfish is a relatively new aquaculture species in countries such Australia, New Zealand, and Mexico. The key attributes of yellowtail kingfish as a

potential aquaculture species include fast growth and increased market demand (Abbink et al., 2012 ; Buentello et al., 2015).

In Japan, this industry primarily is dependent upon the collection of wild juveniles for marine cages farming, although some stocks are supplied through artificial larval rearing from hatcheries (Poortenaar et al., 2001). In Australia, on the other hand, yellowtail kingfish juveniles are produced in hatcheries from closed life cycle reproduction and, thus, aquaculture production does not rely on natural stocks (Chen et al., 2006). In commercial aquaculture, barramundi are exposed to varying water temperatures, ranging from12ºC to 28ºC in Australia (Tanner & Fernandes, 2010), 14ºC to 22ºC in New Zealand (Moran et al., 2009). In fish, fluctuating water temperatures may affect the level of basal metabolism (de Silva, 1995), and thus generate stress (Bowden et al., 2007). However, temperature plays a major role in growth performance and digestive enzyme activities of yellowtail kingfish (Bowyer et al., 2014).

During the grow-out period, yellowtail kingfish are commonly fed with dry extruded diets that contain about 45–50% crude protein, 15–20% crude lipid, 10% ash and 18–20 MJ energy kg^{-1} (Booth et al., 2013). Unlike in South-East Asia where cultured finfish were fed with 'trash' fish, farmed finfish are commonly given dry, pelleted diets (Job, 2011). Department of Fisheries Western Australia (2009) reported that marine finfish are raised on increasingly larger pellets as their growth increases reaching marketable size. Yellowtail kingfish are active predators and normally feed at the water surface (DFWA, 2009 ; Carton & Vaughan, 2010).

FM is the most important protein ingredient in diets for carnivorous marine finfish (Tacon & Metian, 2008 ; Tacon & Metian, 2015), including yellowtail kingfish. Dietary inclusion levels of 35–48% non-genetically modified (GM) varieties of soybean meal (SBM) in yellowtail kingfish resulted in better growth and protein retention compared with those of the fish fed with FM and commercial diets (Buentello et al., 2015). Other studies suggested that yellowtail kingfish can effectively utilised diets containing solvent extracted SBM and soy protein concentrate (SPC) when they were included at the level of 10% and 20%, respectively (Bowyer et al., 2013a ; Bowyer et al., 2013b). In addition, Nguyen et al. (2011) have reported that *S. quinqueradiata*, another closely related species, fed diets

containing soy protein showed comparable growth performance to those fed with FM diets. Given the cost of a formulated diet normally rises as the protein content does, very few efforts have been made to reduce the cost of protein for this species. Studies investigating the effect of inclusion of plant protein in the diets of yellowtail kingfish are therefore necessary.

2.1.2. Barramundi

Barramundi or Asian sea bass (*Lates calcarifer*; Latidae) is a highly valued catadromous fish, originating within the tropical Indo-Pacific region. It is an economically important species and is commercially farmed in Asian countries and Australia (Katersky & Carter, 2005). Barramundi was formerly classified subfamily Latinae in the family Centropomidae, but have since been listed as belonging to the family Latidae (Nelson 1994).

Barramundi possess several attributes as an ideal finfish species for aquaculture. Their hardiness make the barramundi able to tolerate crowding and handling (Ardiansyah & Fotedar, 2016). They have majestic fecundity which allows high and simple seed production in the hatchery (Job, 2011). Moreover, they grow rapidly, achieving a marketable size 350–5 kg in six months to two years (Yue et al., 2009). Barramundi can be reared in marine, brackish and freshwater environments (Kate, 2013). During the grow-out period of the species, net cages or ponds aquaculture are commonly applied in Asia; however, indoor recirculating aquaculture systems are largely used in sub-tropical environments (Job, 2011).

Overall, the maximum growth rate of barramundi is achieved at the temperature between 26 and 33ºC (Katersky & Carter, 2005 ; Katersky & Carter, 2007 ; Williams et al., 2006). However, a study by Bermudes et al. (2010) revealed that temperature ranges for both maximum growth and feed intake were found within the thermal zone for optimal feed efficiency (26ºC–35ºC). Barramundi are able to persist at temperatures below this range; however, below 20ºC growth and metabolism can be deteriorated and fish generally will cease feeding (Job, 2011).

At both commercial and laboratory scales, growth rates varied and have been demonstrated to be influenced by a number of factors including but not limited to

stocking density, grow-out techniques, environmental circumstances, as well as feeding or nutritional-related issues. Optimal protein content specification of dry pelleted diets has been reported to be between 460 and 550 g kg^{-1} (Catacutan & Coloso, 1995 ; Williams et al., 2003a) while requirement for protein is likely to be affected by energy density of the diet and the size of fish used the study (Glencross, 2006). Protein is required by all animals for the regulation of protein synthesis and subsequent structure of the body's tissues and organs.

As with other carnivorous marine finfish, a remarkable growth performance was achieved when the fish are fed with FM-based diet (Boonyaratpalin et al., 1998). As reviewed by Gatlin et al. (2007) and Glencross et al. (2007a), a significant amount of attempts has been made to examine a variety of different ingredients that encompass potential usage in diminishing reliance on FM for use in aquaculture feeds in the past few years. The use of animal or plant-based products (Glencross, 2011 ; Glencross et al., 2011 ; Ngo et al., 2015 ; Williams et al., 2003b) has been the primary focus of the work. It has been shown that either animal meals or plant-based products can function as FM substitutes. However, 15% FM in the diets of barramundi was proposed as a critical threshold when plant protein (PP) sources are included in the diets of this species (Glencross et al., 2011). Total replacement of FM with other protein sources results in poor growth rates in both carnivorous finfish and crustacean species (Kaushik et al., 1995 ; Sharawy et al., 2016).

2.2. Sustainability Issues on Fishmeal Use in Aquaculture Feeds

FM is a widely used protein ingredient in aquaculture feeds, especially those intended for carnivorous marine finfish species (Naylor et al., 2000) (See Figure 2.1). This is due to its outstanding amino acid (AA) profile, high palatability, and excellent nutrient digestibility (Bendiksen et al., 2011 ; Hertrampf & Piedad-Pascual, 2000 ; Huntington & Hasan, 2009). FM are usually sourced of wild-caught, marine bony fish including herring, menhaden, capelin, anchovy, pilchard, sardines and mackerel (Bostock et al., 2010). However, the demand for these small pelagic fish for direct human consumption is huge in Asian countries (Naylor et al., 2000), where the growth of human population is increasing rapidly.

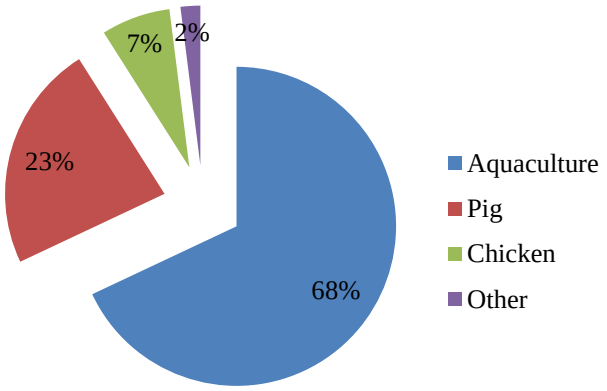

Figure 2.1 Global uses of FM by market segment in 2012 (Tacon & Metian, 2015).

Recently, Tacon and Metian (2015) reported that 70% of aquaculture production come from direct-fed species (35.7 million tonnes in 2012), including tilapia, shrimp, salmon and marine fish, with the remaining 30% being filter-feeding species. Interestingly, 68% of direct-fed species production consumed around 39.6 million tonnes of compound aquaculture feeds, with major consumers being carp (28%), tilapia (18%), shrimp (17%), catfish (11%), salmon (8%), and marine fish (8%) (Tacon & Metian, 2015) (See Figure 2.2). However, as the marine aquaculture keeps expanding, the consumption of FM by carnivorous finfish tend to increase over the next few years (See Figure 2.3), along with its serious implications for the economic viability and health of wild fisheries.

For fisheries, escalating demand and the associated rise in price for fish used in feed production could justify the needs for enhancing sustainable management systems, especially in poorly managed fisheries (Tveterås & Tveterås, 2010). The above scenarios should also offer a tremendous opportunity for both research institutions and industry to reduce the reliance on natural fish stocks by searching sustainable alternatives to FM as the main protein ingredient in aquaculture feeds (Gatlin et al., 2007 ; Huntington & Hasan, 2009 ; Olsen & Hasan, 2012 ; Sarker et al., 2013). Definitely, lower-priced and widely available ingredients that provide a high level of protein, balanced AA content, high digestibility, and appropriate palatability should be in favour of any alternative to FM.

Species group	Demands (MMT)
Fed carp	11.03
Tilapia	6.67
Shrimp	6.18
Catfish	4.27
Marine fish	2.98
Salmon	2.98
Freshwater crustacean	1.8
Freshwater fish (misc)	1.31
Trout	1.14
Milkfish	0.89
Eels	0.37
Total	**39.62**

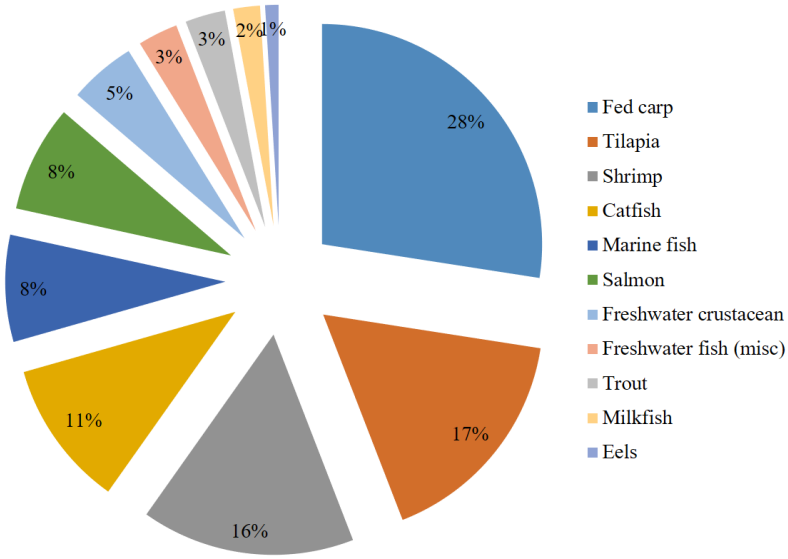

Figure 2.2 Total estimated usage of commercial aquaculture feeds by major fed species in 2012 (Tacon & Metian, 2015).

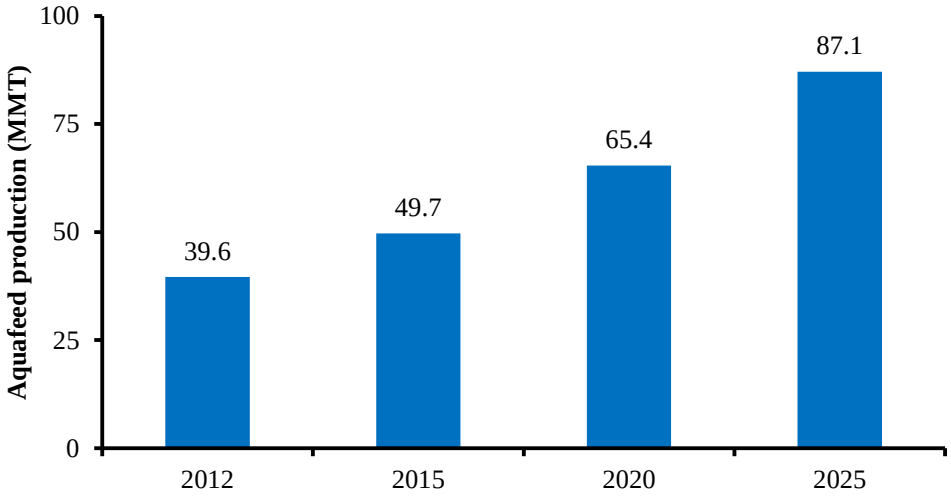

Figure 2.3 Anticipated growth of commercial aquaculture feeds in demand for 2012, 2015, 2020, and 2025 (Tacon & Metian, 2015).

2.3. Plant-derived Protein Ingredients in Aquaculture Feeds

Future growth of aquaculture industry will rely on finding options to substitute FM and FO in aquaculture feeds. Plant protein (PP) sources have been considered as having a great potential to replace marine ingredients in diets for cultured carnivorous finfish species (Bendiksen et al., 2011 ; Glencross et al., 2007a ; Król et al., 2016), due to their favourable nutritional profile relative to FM (Gatlin et al., 2007). PP meals are known to be cost-effective, steadily available, and are a more ecologically friendly protein ingredient than FM (Gatlin et al., 2007 ; Hardy, 2010 ;

Jirsa et al., 2015). Therefore, there have been substantial efforts to boost the amount of PP used in fish feeds in recent years.

Plant-derived sources include soybean meals (Biswas et al., 2007 ; Bonaldo et al., 2006 ; Boonyaratpalin et al., 1998 ; Bowyer et al., 2013a ; Chou et al., 2004 ; Førde-Skjærvik et al., 2006 ; Heikkinen et al., 2006 ; Hernández et al., 2007 ; Kokou et al., 2012) and protein concentrates (Bowyer et al., 2013b ; López et al., 2015 ; Médale et al., 1998), lupin meals (Bórquez et al., 2011 ; Burel et al., 1998 ; Farhangi & Carter, 2001 ; Glencross et al., 2004b ; Glencross et al., 2007c ; Smith et al., 2007a ; Tabrett et al., 2012) and protein concentrates (Glencross et al., 2006b ; Glencross et al., 2010b ; Zhang et al., 2012a ; Zhang et al., 2012b), canola meals (Hussain et al., 2015 ; Mwachireya et al., 1999 ; Ngo et al., 2015 ; Ngo et al., 2016 ; Plaipetch & Yakupitiyage, 2014), cotton seed meals (Barros et al., 2002 ; Cheng & Hardy, 2002 ; Gui et al., 2010 ; Köprücü, 2012 ; Lim & Lee, 2009 ; Rinchard et al., 2003 ; Sun et al., 2015a ; Sun et al., 2015b ; Wang et al., 2014 ; Yue & Zhou, 2008), corn meals (Güroy et al., 2013 ; Jahanbakhshi et al., 2013 ; Regost et al., 1999 ; Sevgili et al., 2015), pea meals and concentrates (Allan & Booth, 2004 ; Bautista-Teruel et al., 2003 ; Borlongan et al., 2003 ; Collins et al., 2012 ; Davies & Gouveia, 2008 ; Gouveia & Davies, 2000 ; Penn et al., 2011 ; Zhang et al., 2012a), faba bean meals (Azaza et al., 2009 ; Ouraji et al., 2013 ; Soltanzadeh et al., 2015), and copra meals (Mukhopadhyay & Ray, 1999).

Numerous works demonstrating a wide range of feed ingredients, feed formulations and experimental methods have been completed to study the effects of the inclusion of a non-FM dietary component derived from plant protein (PP) sources on fish growth and health status. In general, alternative PP sources can be included in diets at relatively low levels (0-50%) without hampering growth, as reported by many authors (Adelizi et al., 1998 ; Bonaldo et al., 2011 ; Carter & Hauler, 2000 ; Collins et al., 2012 ; Güroy et al., 2013 ; Hixson et al., 2016 ; Jirsa et al., 2015 ; Pratoomyot et al., 2010 ; Sun et al., 2015b ; Wang et al., 2016b). However, particularly for carnivorous species, diets containing high level (50-100%) of PP materials can cause the impairment of growth performance and health, which is commonly associated with issues concerning the undesirable qualities of PP ingredients including low palatability, imbalanced AA, and the presence of

antinutritional factors (ANF) (Chou et al., 2004 ; Farhangi & Carter, 2001 ; Glencross et al., 2004a ; Hernández et al., 2007 ; Peng et al., 2013 ; Tantikitti et al., 2005 ; Wang et al., 2006b ; Zhou et al., 2005).

ANF induce a limiting effect on protein and mineral utilisation by fish, which may lead to growth retardation and undesirable health condition (Francis et al., 2001). Intestinal inflammatory found in soybean meal (SBM)-fed rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) (Krogdahl et al., 2003 ; Ostaszewska et al., 2005) was caused by ANF. In addition, due to ANF-mineral complexities, PP sources are known to insufficiently provide micronutrients required by fish for normal growth and development (Kumar et al., 2012). However, limited information is available on the effects of dietary PP sources lacking micro-minerals in carnivorous finfish.

Furthermore, since tolerance to dietary PP is known to be species- and size-specific (NRC, 2011), cautious assessment of available data must be taken when formulating diets to ensure the provision of species-specific and size-specific feeds that satisfy the nutritional requirement of the farmed species can be achieved (Vielma et al., 2000). Sufficient information of mineral utilisation at relevant environmental conditions is also essential for enhancing both dietary composition and feeding strategies.

2.3.1. Soybean meal

Soybean (*Glycine max* Linnaeus) is one of the most prominent crop worldwide (Asbridge, 2015). The methods used to produce various soybean products are shown in Figure 2.4. Soybean meal (SBM) is the by-product from the extraction of soy oil (Gao et al., 2013). SBM is the most utilised PP product in aquaculture feeds (NRC, 2011) Compared to other PP sources, SBM is considered as the most lucrative FM substitute to be included in diets for many aquaculture species, on account of its relatively balanced AA profile, high protein content, favourable digestibility, cheaper price, and reliable supply relative to FM (Gatlin et al., 2007). SBM contains 43-53% crude protein, 1.8-2.4% crude lipid, and 18-20 MJ kg^{-1} gross energy (Bowyer et al.,

2013a ; Nengas et al., 1996 ; Sevgili et al., 2015), ideal for a wide array of fish species.

The application of SBM as an alternative protein for FM has been appraised for many commercially important fish and crustacean species, such as Japanese seabass (*Lateolabrax japonicus*) (Zhang et al., 2014a), gilthead sea bream (*Sparus aurata*) (Nengas et al., 1996 ; Venou et al., 2006), Japanese flounder (*Paralichthys olivaceus*) (Kikuchi, 1999), Korean rockfish (*Sebastes schlegeli*) (Lim et al., 2004), Atlantic cod (*Gadus morhua*) (Refstie et al., 2006a), red drum (*Sciaenos ocellatus*) (Rossi Jr et al., 2015), Australian red claw crayfish (*Cherax quadricarinatus*) (Muzinic et al., 2004), and kuruma shrimp (*Marsupenaeus japonicus*) (Bulbul et al., 2013). These studies uncovered that FM can be successfully replaced by SBM without negatively influencing growth and health of the fish.

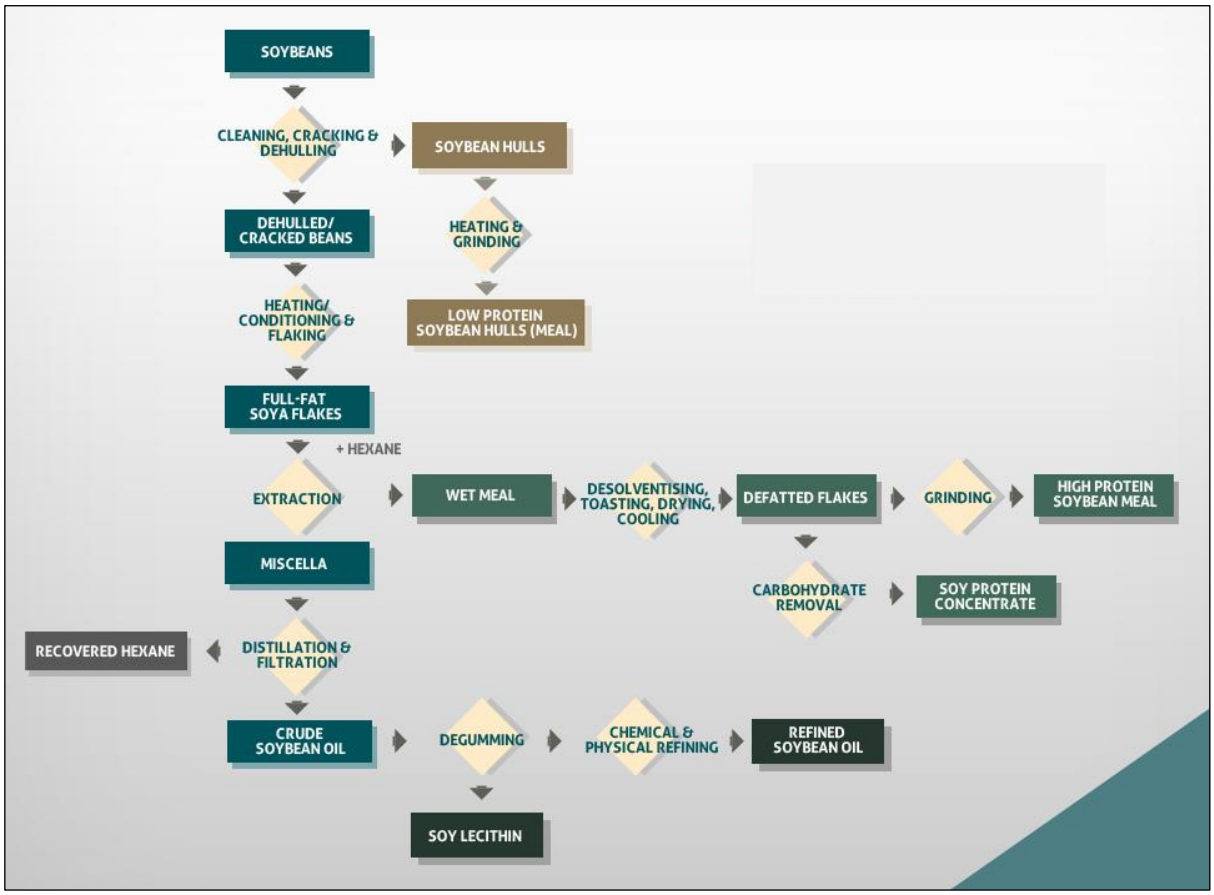

Figure 2.4 SBM production process (www.soymeal.org)

On the other hand, SBM contain some antinutrient components including oligosaccharides, lectins, saponins, tannins, trypsin inhibitors, and phytic acid (Francis et al., 2001), which possess a depressive effect on feed utilisation by interfering with the digestion and nutrient absorption of nutrients (Krogdahl et al., 2010). While oligosaccharides, lectins, saponins, tannins, and trypsin inhibitors can be inactivated by heat treatment or alcohol extraction during manufacture processes, phytate cannot be degraded by any of these techniques (Francis et al., 2001 ; Krogdahl et al., 2010 ; Raes et al., 2014 ; Reddy & Pierson, 1994). Phytic acid or phytate (myo-inositol hexakisphosphate, IP6), is a common constituent of PP seeds, making up around 50–80 % of the total phosphorous (P) (Kumar et al., 2012). This phytate-bound P cannot be utilised by monogastric animals, including fish (Usmani & Jafri, 2002). The antinutritional feature of phytate is compelled by the strong chelation of phytate with divalent minerals, such as copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg), calcium (Ca) and selenium (Se) (Sathe & Reddy, 2002). Inhibition of growth, feed utilisation and protein and mineral bioavailability was reported in fish fed diets containing high amounts of phytate (Storebakken et al., 1998). The effect of phytate-containing PP-based diets on performance of carnivorous marine finfish is not well-established.

2.3.2. Lupin meal

Lupins (*Lupinus* spp.) are non-starch legumes in the family Fabaceae that possess excellent potentials as sources of protein in diets for marine finfish (Glencross et al., 2010a ; Zhang et al., 2012b). Many species of lupins are cultivated in many countries, but only four serve as suitable ingredients in animal feeds, namely *L. albus*, *L. angustifolius*, *L. luteus* and *L. mutabilis* (Glencross & Hawkins, 2004 ; Glencross et al., 2010a). Interestingly, around 75% of global lupin production are sown in Australia (Lucas et al., 2015), with Western Australia being the major producers (Chen et al., 2015). The lupin products included in aquaculture feeds are meal derived from whole or dehulled seed (lupin kernel meal). Lupin meal (LM, *L. angustofolius*) have a proven potential as an alternative to FM due to its comparatively high protein quality, good palatability, outstanding digestibility, lower cost and consistent availability (Gatlin et al., 2007). Typically, in the kernel form,

LM have ~28-42% crude protein, 0.06-0.1% crude lipid content, and 19 MJ kg^{-1} gross energy (Petterson, 2004).

A substantial amount of FM in the fish diet can be successfully replaced with LM a range of omnivorous species, such as tilapia (*Oreochromis niloticus* × *O. aureus*) (Chien & Chiu, 2003), silver perch (*Bydianus bidyanus*) (Allan & Booth, 2004), and black tiger shrimp (*Penaeus monodon*) (Smith et al., 2007a ; Smith et al., 2007b ; Sudaryono et al., 1999). Previous studies with rainbow trout (*Oncorhynchus mykiss*) showed that LM can be included in the diet without a deleterious effect on nutrient utilisation, growth and health condition (Farhangi and Carter, 2001, 2007; Glencross and Hawkins, 2004; Glencross et al., 2008). Similarly, desirable growth and health performance was found in Atlantic salmon (*Salmo salar*) (Salini & Adams, 2014), black sea bream (*Acanthopagrus schlegeli*) (Zhang et al., 2012b), and snapper (*Pagrus auratus*) (Glencross et al., 2003b ; Glencross & Hawkins, 2004). These indicates that LM is also a feasible source of protein for carnivorous marine species.

However, retarded growth is evidenced in rainbow trout fed diets containing 50% dehulled LM (Farhangi & Carter, 2001). In addition, results have shown that the amount of LM that is preferred in the rainbow trout (*O. mykiss*) diets are affected by alkaloids, oligosaccharide and non-starch polysaccharides (NSP) content (Francis et al., 2001 ; Glencross, 2009 ; Glencross et al., 2003a). Moreover, similar to most other vegetable feed ingredients, LM also contain other ANFs which may have adverse effects on both their nutritional value and palatability. In addition to other important ANFs such as protease inhibitors, saponins, phytoestrogens, alkaloids, phytate one of the most noticeable ANFs in LM (Francis et al., 2001 ; Kumar et al., 2012). Treatments involving heat, alcohol or aqueous extraction during production process can degrade protease inhibitors, saponins, phytoestrogens, alkaloids in the PP ingredient (Krogdahl et al., 2010). In the other hand, phytate is heat-stable (Hardy & Barrows, 2002) and forms insoluble complexes with nutritionally essential minerals such as copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), manganese (Mn), iron (Fe), magnesium (Mg), calcium(Ca) and selenium(Se) (Connelly, 2011). Roughly 50% of total phosphorus (P) in LM is bound as phytate (Kumar et al., 2012) and the P moiety of phytate is inadequately available to fish (Storebakken et al., 1998). As with SBM, information concerning the importance of dietary minerals for fish fed LM-

containing diets is very limited. In this view, attempts to formulate nutritionally-improved PP-based diets with diminished phytate levels and thus, improve mineral bioavailability remains pivotal.

2.4. Enhancement of Plant Protein Products

To allow increased inclusion of PP ingredients in fish diets, efforts are directed at the improvement of the nutritional quality of through further processing. Moist or dry heating during extraction process of PP feedstuffs, to some extent, were able to degrade the content of ANFs in legume seeds, especially trypsin inhibitor, tannin, oligosaccharides, phytate, and phenolic compounds (Drew et al., 2007 ; Khattab & Arntfield, 2009 ; Raes et al., 2014). Glencross et al. (2007b) reported that the utilisation of digestible protein and energy of the ingredient by fish is improved when heat-treated PP ingredients are included in the diet. In Korean rockfish (*Sebastes schlegeli*), dietary incorporation of unprocessed SBM is limited to 20% of the dietary protein (Lim et al., 2004). What is more, the digestibility of many PP feedstuffs seems to be unfavourable due to the limited availability of enzymes required to shatter the complex cell wall structure that compresses other nutrients (Castillo & Gatlin, 2015).

Ingredients processing through mechanical treatment, soaking, germination, enzyme treatment, and fermentation is also applicable to improve the nutritional value of plant feedstuffs (Raes et al., 2014). A solid state fermentation process had a major effect in dephytination and reduction of tannins in LM (Van Vo et al., 2015). Azarm and Lee (2014) described that the nutritional quality of SBM could be improved by fermentation with *Bacillus subtilis*. Through fermentation with *B. subtilis*, the antinutritional properties of cottonseed meal (CSM) were significantly reduced (Sun et al., 2015a). Fermentation with baker's yeast *Saccharomyces cerevisiae* could also improve the nutritive value of PP products, as suggested by Hassaan *et al.* (2015). Degradation of phytic acid content was found during microbial fermentation of LM (Fritsch et al., 2015). Moreover, fermentation of vegetable product was reported to enhance phagocytic activity and superoxide production of leukocytes in Japanese flounder (*Paralichthys olivaceus*) (Ashida & Okimasu, 2005).

An additional constraint to the formulation of PP-based diets in carnivorous marine finfish diets is that the trace mineral concentration of PP sources is reduced much below the optimum level due to the complexing actions of phytate (Francis et al., 2001 ; Krogdahl et al., 2010 ; Sajjadi & Carter, 2004). Therefore, another possible method to improve the quality of PP sources is the exogenous supplementation of trace minerals. It is well known that trace minerals (Cu, Fe, Se, and Zn, among others) are vital for maintaining all physiological and biochemical functioning (López-Alonso, 2012 ; Watanabe et al., 1997). Although a mineral premix is commonly added in the preparation of fish diets, levels of minerals in the premix are formulated for FM-based diets (Welker et al., 2015), and may be inadequate for fish fed PP-based diets.

2.5. Importance of Se in Aquaculture

The element of Se was discovered in 1817 and it is an essential micronutrient for all animals, including aquatic animals (McKenzie et al., 2002). In fish, it is required for the effective operation of normal growth, physiological function, cellular metabolism and immune response (Gatlin et al., 1986 ; Watanabe et al., 1997). Biological essentiality of Se was first described by Schwarz and Foltz (1957), before unequivocal evidence of a physiological function of Se as a component of the enzyme glutathione peroxidase (GPx) was established (Rotruck et al., 1973). This GPx is important because it catalyses reactions essential for the transformation of potentially damaging fatty acid hydroperoxides and hydrogen peroxide into water and fatty acid alcohol, thus providing protection at both cellular and subcellular level against oxidative damage (Arthur et al., 2003). However, Se status of fish affects the activity level of the GPx in liver or plasma.

Uptake of Se by fish can occur through two processes: direct uptake of waterborne Se across the gills or epidermal surfaces, and uptake of foodborne Se (Ogle et al., 1988). However, dietary exposure to Se compounds is normally the principal pathway of Se accumulation in fish (Hamilton, 2004 ; Janz, 2011). Tissue Se concentration in beluga (*Huso huso*) and gibel carp (*Carassius auratus gibelio*) reflected the Se concentration of diets supplemented with Se (Arshad et al., 2011 ; Han et al., 2011). Tissue Se content and distribution ranges somewhat among various experiments, with the spleen, liver, and kidney are the primary sites of accumulation

while muscle tissue seems to accumulate the least (Han et al., 2011 ; Le & Fotedar, 2014c ; Liu et al., 2010 ; Lorentzen et al., 1994 ; Misra et al., 2012 ; Wang & Lovell, 1997). Therefore, the fraction of accumulated Se in the edible tissue of fish presents little risk for human consumption.

There are two major sources of Se for fish: inorganic (i.e. sodium selenite, selenate) and organic (i.e. selenized yeasts, seleno-methionine) sources. It has been confirmed that, with regard to digestibility, accumulation and biological availability, organic forms of Se are better than its inorganic forms (Cotter et al., 2008 ; Fontagne-Dicharry et al., 2015 ; Jaramillo Jr et al., 2009 ; Kucukbay et al., 2009 ; Le & Fotedar, 2014a ; Wang et al., 2007). However, Se nanoparticles (nano-Se) have attracted growing attention due to their great potential in improving growth and physiological performance of fish (Zhou et al., 2009).

Se has been discovered to be demanded in the diet of many fish species. The Se requirements of fish vary with the form of Se ingested, polyunsaturated fatty acid (PUFA) and vitamin E content of the diet, and the concentration of waterborne Se (NRC, 2011). Diets deficient or excessive in Se can manifest in a range of nutrition-related syndromes, i.e. diminished growth, reduced plasmid and tissue GPx activity, proliferated lipid peroxidation, and tissue relapse (Ashouri et al., 2015 ; Cleveland et al., 1993 ; Cotter et al., 2008 ; Dörr et al., 2008 ; Fontagne-Dicharry et al., 2015 ; Gatlin et al., 1986 ; Hamilton et al., 1990 ; Hilton et al., 1980 ; Jaramillo Jr et al., 2009 ; Wang et al., 2013 ; Zhu et al., 2012). It has been suggested that marine fish require a high amount of PUFA in their diets, which potentially lead to increased tissue lipid peroxidation and decreased antioxidant activity, due to the conversion of PUFA to toxic peroxides (Webster & Lim, 2002). In this view, dietary Se is required to activate the enzyme GPx to prevent these detrimental effects. However, limited information is available on the Se requirements of marine finfish species.

2.6. Summary

Due to limited supply and rising demand of FM, nutritionists should find approaches to reducing the cost of feeding in aquaculture. The inclusion of plant-protein sources in diets of carnivorous species is one of the greatest challenges for the sustainability of aquaculture industry. Studies concerning the use of plant-derived protein

ingredients such as SBM and LM in fish and crustacean have accumulated rapidly, but information on the effects of these mineral-deficient diets on growth and health performance is scanty. Therefore, research is needed to elucidate the roles of dietary minerals including Se in enhancing growth and health performance of cultured fish.

CHAPTER 3: Growth, antioxidant capacity and muscle histochemistry of yellowtail kingfish (*Seriola lalandi* Valenciennes 1883): selenium and temperature interaction

This research is published in Animal Feed Science and Technology
Volume 217 (2016): 76-86

3.1. Introduction

Disease continues to be the major constraint in the development of sustainable aquaculture. Elevated stress levels are the root of most diseases in farmed fish (Turnbull, 2012 ; Wedemeyer, 1997). A number of studies have shown that increased disease susceptibility and reduced immune response were attributable to acute or chronic stress responses (Barton & Iwama, 1991 ; Dang et al., 2012 ; Davis et al., 2002 ; Dietrich et al., 2014 ; Fridell et al., 2007 ; Iguchi et al., 2003 ; Li et al., 2014 ; Nakano et al., 2014 ; Small & Bilodeau, 2005 ; Tacchi et al., 2015 ; Varsamos et al., 2006). Thus, in most cases diseases infect farmed fish following exposure to stress condition.

It is well recognised that fish previously exposed to a stressor may show oxidative stress response (Lushchak & Bagnyukova, 2006), which is a reflection of an imbalance between the levels of prooxidant and antioxidant properties (Sies, 1985). Prooxidant substances include those relating to reactive oxygen species (ROS), chemically reactive molecules containing oxygen, which are responsible for lipids, proteins and deoxyribonucleic acid (DNA) impairment, particularly when fish lack the capacity to deal with accumulated ROS production (Vinagre et al., 2012). Antioxidant properties are classically defined as any substance that provides protection against oxidative damage (Pamplona & Costantini, 2011). The equilibrium between ROS production and antioxidant stores that the fish maintain, therefore, regulate the extent to which oxidative damage can occur in fish. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) establish a fundamental aspect of the antioxidant response (Winston, 1991).

Selenium (Se), an essential micro-mineral required for maintaining the growth and metabolic function of fish, serves as an integral structural element of the active core of GPx enzymes in red blood cells (RBCs) (Rotruck et al., 1973). Although Se is needed only in trace amounts, Se engrosses a distinctive attention among micro-minerals as the constraint in the antioxidant enzyme biosynthesis. Se-containing GPx plays an important role in protecting cells and membranes from oxidative stress by catalysing the reduction of hydrogen peroxide and lipid peroxides using reduced glutathione (GR) (Watanabe et al., 1997). The activity of this enzyme is modulated by sufficient Se intake (Dhur et al., 1990) and it is well known that, although fish can accumulate Se via both surrounding water and food, dietary exposure to Se compounds comprises the predominant source of Se for fish (Janz, 2011).

Dietary supplementation of Se has been reported to enhance the antioxidant enzyme capacity precursor in crayfish *Procambarus clarkii* (Dörr et al., 2008), freshwater prawn (*Macrobrachium rosenbergii*) (Chiu et al., 2010), whiteleg shrimp (*Litopenaeus vannamei*) (Parrilla-Taylor & Zenteno-Savín, 2011 ; Parrilla-Taylor et al., 2013), common carp (*Cyprinus carpio*) (Elia et al., 2011), crucian carp (*Carassius auratus gibelio*) (Zhou et al., 2009), rainbow trout (*Oncorhynchus mykiss*) (Kucukbay et al., 2009), and cod (*Gadus morhua*) (Penglase et al., 2010). An additional benefit of using Se as a feed supplement is that elevated dietary levels improve growth and feed utilisation in a variety of aquatic species, including African catfish (*Clarias gariepinus*) (Abdel-Tawwab et al., 2007), gibel carp (*C. auratus gibelio*) (Han et al., 2011), and abalone (*Haliotis discus hannai* Ino) (Wang et al., 2012). In most fish, Se deficiency leads to poor growth performance, lipid peroxidation and decreased GPx enzymatic activity (Bell et al., 1986 ; Hilton et al., 1980 ; Wang et al., 2007).

Se exists in two forms, namely inorganic Se (selenate and selenite) and organic Se (selenomethionine, Se-met and selenocysteine, Se-cys) (Sunde, 2006). The amount of dietary Se demanded to enhance growth and antioxidant capacity is dependent on Se sources. For instance, using sodium selenite, Hilton et al. (1980) recommended that a concentration of 0.38 mg Se kg^{-1} is suitable for rainbow trout (*O. mykiss*). With the similar form of Se, Gatlin and Wilson (1984) demonstrated that the optimum dietary supplementation of Se for channel catfish (*Ictalurus punctatus*) is

0.25 mg Se kg^{-1}. However, in an investigation employing Se-met, the juvenile grouper (*Epinephelus malabaricus*) required 0.77 mg Se kg^{-1} for their best growth performance (Lin & Shiau, 2005). Han *et al*. (2011) found that the dietary Se-met requirement for gibel carp (*C. auratus gibelio*) is 1.18 mg Se kg^{-1}. Another experiment on African catfish (*C. gariepinus*), conducted by Abdel-Tawwab et al. (2007), showed that a Se-met concentration of 3.67 mg Se kg^{-1} was proposed as the optimum level needed to improve fish growth and vitality against environmentally-induced stress.

Se nutrition of yellowtail kingfish (*Seriola lalandi*), an emerging species in Australia and New Zealand aquaculture, has been recently studied. Le and Fotedar (2014a) reported that Se-met and seleno-yeast were the most bioavailable sources of Se to yellowtail kingfish. Se from fishmeal (FM)-based diets was inadequate to maintain the growth performances of the species and therefore supplementation with organic Se was recommended. In addition, dietary Se significantly improved yellowtail kingfish survival, antibodies, and haematocrit, following exposure to bacterial challenge, and the role of Se as an antioxidant was established by activities such as resistance of RBCs to peroxidation and GPx (Le & Fotedar, 2014b). However, very limited data and information is available on the effects of temperature on micro-minerals such as Se utilisation, which may in turn, influence the growth and health of the cultured species.

In commercial aquaculture, yellowtail kingfish are exposed to fluctuating water temperatures, which can affect the level of basal metabolism (de Silva, 1995), and thus induce stress (Bowden et al., 2007 ; Nakano et al., 2014). The species are presently cultured in sea cages at temperatures ranging from 12°C to 28°C in Australia (Tanner & Fernandes, 2010) and 14°C to 22°C in New Zealand (Moran et al., 2009). Therefore, this study was designed to investigate the effect of organic Se supplementation on growth as well as health performance of juvenile yellowtail kingfish at two temperatures that represent ambient and elevated water temperatures. Adequate knowledge of mineral utilisation at relevant environmental temperatures is important for optimising dietary composition and feeding conditions (Kim et al., 2006), thus, improving fish performance under culture situations, particularly during the grow-out phase.

3.2. Materials and methods

3.2.1. Experimental fish and diets

Hundred eighty healthy yellowtail kingfish juveniles (58.17 g) were provided by the Australian Centre for Applied Aquaculture Research (ACAAR) Laboratory, at the Challenger Institute of Technology (Fremantle, Western Australia), where this study was carried out. Yellowtail kingfish juveniles were acclimated to laboratory conditions and fed the control diet twice daily at 4.75% body weight for one week prior to the commencement of experiment.

A basal mash of a commercially available yellowtail kingfish diet without any supplementation of organic Se (OS) was used to prepare the experimental diets. Three isonitrogenous and isocalorific experimental diets were prepared and differed only by the supplementation level of OS (Sel-Plex®, Alltech Inc., Lexington, Kentucky, USA) at 0.0 (control), 2.0, or 4.0 mg Se kg^{-1} diet. As FM-based diets contained around 3.40 mg Se kg^{-1}, the actual concentrations of Se were 3.35 mg Se kg^{-1}, 5.46 mg Se kg^{-1} and 7.38 mg Se kg^{-1} for three experimental diets. The experimental diets, with an approximate chemical composition of 46.42% of protein, 15.05% of lipids, 9.56% of ash, 91.58% of dry matter and 40 mg kg^{-1} of vitamin E, were prepared at the Australasian Experimental Stockfeed Extrusion Centre (Roseworthy, Adelaide, Australia) as cooked, extruded, slow-sinking 3 mm pellets.

Fish handling procedures, care, and facilities complied with the guidelines of the Animal Ethics Committee of Curtin University and followed the Australian Code of Practice for the care and use of animals for scientific purposes.

3.2.1. Experimental design

Two similar flow-through systems were designed to raise fish at 21°C and 26°C water temperatures. Both systems were located indoors and were set up as two identical flow-through systems with a total volume per system of 1,800 L. Each system was equipped with nine cylindrical tanks (200 L each), a submersible water pump, and aeration. An air diffuser, central drain and sponge filter were installed in each tank to allow for oxygenation, waste removal and biological filtration, respectively. The water flow rate in each system was adjusted to 4.8 L min^{-1}, to maintain dissolved oxygen above 80% saturation. A 1,000-W titanium heater and

digital controller (WEIPRO MX-1019) were set to maintain a stable water temperature. The water temperatures measured in the experimental tanks generally followed the two temperature regimes targeted in the study design as values from daily manual measurements in each tank were in agreement with records from the temperature digital controller.

Throughout the experiment, the water quality parameters of the rearing water were monitored daily. Dissolved oxygen was monitored by using a DO meter (CyberScan DO 300, Eutech Instruments, Singapore). Ammonia and pH were measured every two days using chemical test kits (Aquarium Pharmaceuticals Ltd, UK) and a pH meter (CyberScan pH 300, Eutech Instruments, Singapore). Water salinity was maintained every day between 34-35 ppt, and measured by a portable refractometer (RHS-10ATC).

Following acclimation, animals were transferred and reared in 21°C and 26°C treatment tanks, respectively, where yellowtail kingfish were stocked at ten fish per tank in triplicates. Upon commencement of the experiment, the animals were physically inspected, and the initial weights of animals from each dietary treatment were recorded. Fish were fed to apparent satiation twice daily, at approximately 09:00 and 14:00 hrs. The feeding trial lasted for 30 days.

3.2.3. Sampling and analytical methods

The proximate composition, including crude protein, crude ash, gross energy and moisture content of basal diets, was determined following the protocol established by the Association of Official Analytical Chemists (AOAC, 1990). Crude protein was analysed by using the Kjeldahl method, with a Kjeltec Auto 1030 analyser; lipids, by extraction with petroleum ether using a Soxtec System; moisture, by drying at 105°C in an oven (Thermotec 2000, Contherm Scientific, Hutt, New Zealand) to a constant weight; and ash, by combustion at 550°C for 24 hours in an electric furnace (Carbolite, Sheffield, UK).

Throughout the trial, the growth performance indicators that were measured were the specific growth rate (SGR, % day^{-1}), relative feed intake (RFI, g day^{-1}), and survival (%), according to the following formulae:

$$SGR=(\ln W_2 - \ln W_1)t^{-1} * 100$$

where W_1 and W_2 are the initial and final weights, respectively; t is the number of days in the feeding trial (Sveier & Lied, 1998).

$$RFI=100*(\text{dry feed intake})*\left[\frac{BW_1+BW_2}{2}\right]^{-1} *(\text{days fed})^{-1}$$

where BW_1 and BW_2 are the initial and final body weights, respectively (Grisdale-Helland et al., 2008).

$$\text{Survival (S, \%)} = 100 \times \left[\frac{\text{final number of fish}}{\text{initial number of fish}}\right]$$

At the end of the feeding trial, following a 24-hour fast, six fish from each tank were selected and anaesthetised with tricaine methane sulphonate (MS222) (50 mg L^{-1}) for final weighing and blood collection.

3.2.4. Haematology and osmolality

Fish blood samples from three fish $tank^{-1}$ were drawn by caudal vein puncture, with a 1-mL plastic syringe. The extracted blood was divided into two sets of tubes. The first set contained K_2EDTA (BD Vacutainer® Plus Plastic), used as an anticoagulant, for haematology while the second set (Eppendorf tubes) was left without an anticoagulant, for osmolality. Hb kit (Randox Laboratories, Antrim, United Kingdom) was used to measure the haemoglobin (Hb). For separation of serum, blood samples were immediately transferred to a 1 mL vial and allowed to clot at 4^oC, in a refrigerator. The blood was centrifuged for 30 minutes at 5,000 rpm (Eppendorf 5430 R, Eppendorf Ltd., Hamburg, Germany). The serum was finally transferred to a 0.5 mL vial for osmolality analysis in a cryoscopic osmometer– Osmomat 030-D (Genotec).

3.2.5. Histopathology assay

After blood sampling, left anterior dorsal muscle from three euthanised fish $tank^{-1}$ were quickly dissected and prepared for histological examination. Light microscopy samples were prepared using standard histological techniques (Luna, 1968). Tissue samples were fixed in 4% saline, formalin dehydrated in ethanol, before equilibration in xylene and embedment in paraffin wax. Sections of approximately 5 µm were cut

and stained with haematoxylin and eosin for histological examination, under an Olympus BX40F4 light microscope.

3.2.6. Antioxidant glutathione peroxidase assay

Erythrocytes (red blood cells)' GPx activities were quantitatively assayed by using the Randox Laboratories test combination (Ransel, Antrim, United Kingdom). According to the method of Paglia and Valentine (1967), GPx catalyses the oxidation of glutathione using cumene hydroperoxide in the presence of glutathione reductase (GR) and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The oxidised glutathione (GSSG) was immediately converted to the reduced form, with a concomitant oxidation of NADPH to nicotinamide adenine dinucleotide phosphate ($NADP^+$). The decrease in absorbance at 34 nm was measured at 37°C.

3.2.7. Selenium analysis

The Se concentration of muscle tissue and experimental diets were determined by the Marine and Freshwater Research Laboratory, Environmental Science, Murdoch University (Western Australia) based on the method of inductively coupled plasma atomic emission spectrometry (Agilent 720 ICP-AES). The detection limit for Se through this method is approximately 0.02 to 50 ppm. All Se levels for diet and tissue samples are recorded as dry weights.

3.2.8. Statistical analysis

All data were expressed as mean ± standard error (SE). The effects of dietary organic Se supplementation (3 levels: 0.0, 2.0 or 4.0 mg Se kg^{-1} diet) and rearing temperature (2 levels: 21°C or 26°C) on growth performance, feed utilisation, survival, osmolality, plasma GPx, and Se concentration were analysed with the two-way analysis of variance (ANOVA). Survival data were arcsine transformed. If a significant interaction was detected between the main variables, then the variable was tested using a one-way ANOVA. The Duncan method was applied to determine significant differences among treatment groups, and probability values $\rho<0.05$ indicate a significant difference. All statistical analyses were performed using the IBM SPSS Statistics 22 (Australia).

3.3. Results

Final weight (FW), specific growth rate (SGR), relative feed intake (RFI), survival and osmolality of juvenile yellowtail kingfish reared at two temperatures (21°C and 26°C) and fed the diets containing different levels of organic Se are presented in Table 1. FW and SGR were significantly affected by dietary Se level ($P < 0.001$) and water temperature ($P < 0.001$), and an interaction effect was present ($P < 0.001$). The significant interaction was reflected by a greater increase in FW and SGR, in fish supplemented with 2.0 mg Se kg^{-1} at 21°C than at 26°C ($P < 0.001$). There was no significant interaction between temperature and diet, with regards to RFI, and it was significantly affected by Se levels in the diet ($P = 0.024$). Fish that were fed diets supplemented with 2.0 and 4.0 mg Se kg^{-1}, attained lower RFI than those fed the control diet. The RFI of fish reared at 21°C and 26°C was similar ($P > 0.05$). The survival of the juvenile yellowtail kingfish ranging from 90% to 97% was not significantly different among any treatment groups over the course of the feeding experiment. No significant interaction was found between temperature and diet ($P > 0.05$) on fish osmolality. However, osmolality was significantly influenced by temperature, as higher osmolality (311 mOsm kg^{-1}) was observed in fish reared at 26°C ($P < 0.001$).

The erythrocyte GPx of juvenile yellowtail kingfish at 21°C was significantly different between Se unsupplemented and supplemented dietary treatments ($P < 0.05$), which were 83.73±2.87 U g^{-1} Hb (control diet), 97.27±2.06 U g^{-1} Hb (2.0 mg Se kg^{-1}), and 100.63±3.27 U g^{-1} Hb (4.0 mg Se kg^{-1}) (Figure 3.1). A similar trend was observed in the erythrocyte GPx of fish reared at 26°C. However, the GPx activity of fish that were fed Se-supplemented diets was highest at 26°C ($P < 0.05$), ranging from 106.33±1.87 to 109.60±3.16 U g^{-1} Hb.

Table 3.1. Growth performance, relative feed intake (RFI), survival and osmolality of juvenile yellowtail kingfish fed with and without Se supplementation at two different temperatures[1]

Temperature	21°C			26°C			Analysis of variance[2]				
Sel-Plex® (mg kg^{-1})	0	2	4	0	2	4	Temp 21°C vs 26°C	Se level 0	2	4	Interaction
IW	68.0±2.08	69.0±3.03	64.4±2.25	66.7±0.92	68.3±1.09	64.7±1.58					
FW	101.1±2.75^{a}	128.7±4.05^{c}	113.3±1.5^{b}	86.1±0.64^{a}	90.7±1.48^{a}	101.7±1.6^{b}	>		*		*
SGR	1.37±0.04^{a}	2.20±0.06^{b}	1.99±0.11^{b}	0.85±0.02^{a}	0.99±0.01^{b}	1.51±0.05^{c}	>			*	*
RFI	1.04±0.12	1.32±0.42	1.22±0.26	1.02±0.18	1.12±0.21	1.05±0.37	ns	x	y	y	ns
Survival	93±1.08	97±0.85	93±1.08	90±1.32	93±0.85	90±1.08	ns	ns			ns
Osmolality	293±1.52	298±0.57	299±4.35	307±1.52	310±0.57	315±1.15	<	ns			ns

[x,y,z]For variables with a significant effect of diet and no significant interaction, values without a common letter are different (z indicated the highest value; $P < 0.05$).

[a,b,c]For parameters with a significant interaction, differences in diets are compared within each temperature ($P < 0.05$), and values without a common superscript are different.

[1]Means of three replicates ± SE.

[2]ns, non significant; *, $P < 0.05$. For variables with a significant effect of temperature ($P < 0.05$), and no interaction, < or > indicates whether the values measured at 21 °C were less than or greater than those measured at 26 °C.

IW (final weight, g); FW (final weight, g); SGR (specific growth rate, % body weight day^{-1}); RFI (relative feed intake, g fish^{-1} day^{-1}); S (survival, %)

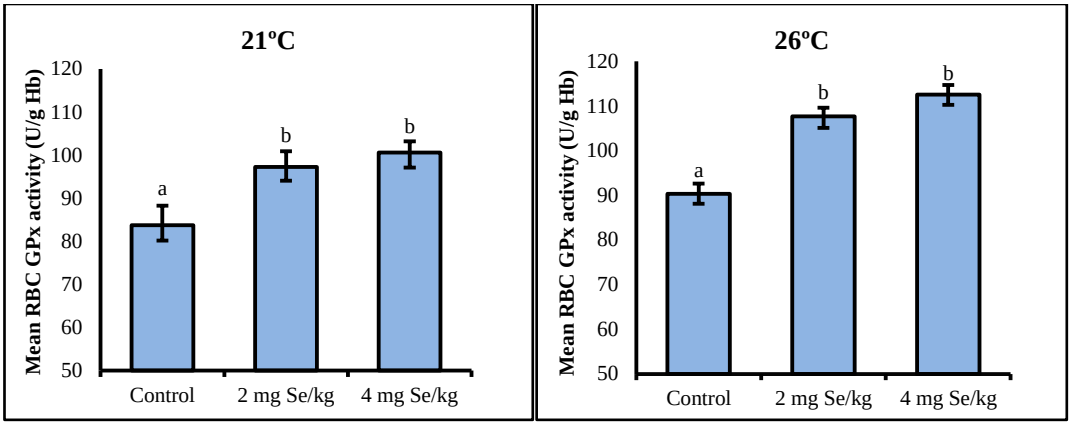

Figure 3.1. Plasma glutathione peroxidase (GPx) activities of juvenile yellowtail kingfish fed diets containing different Se supplementation levels at 21°C and 26°C temperatures.

After one month of the feeding trial, muscle Se concentrations of fish that were fed different levels of Se supplementation at 21°C were significantly different. At 21°C, muscle Se content in fish generally increased, as the dietary Se supplementation increased. Muscle Se concentrations of fish that were fed the control, 2.0 mg Se kg^{-1} and 4.0 mg Se kg^{-1} supplementation diets were 1.47±0.10 mg kg^{-1}, 2.33±0.10 mg kg^{-1} and 2.86±0.03 mg kg^{-1} dry weight, respectively ($P < 0.001$). In contrast, when fish were reared at 26°C, the muscle Se concentration of those fed a diet supplemented with a 4.0 mg Se kg^{-1} diet was significantly higher ($P < 0.05$), while there was no difference between the control and 2.0 mg Se kg^{-1} dietary treatments (Figure 3.2).

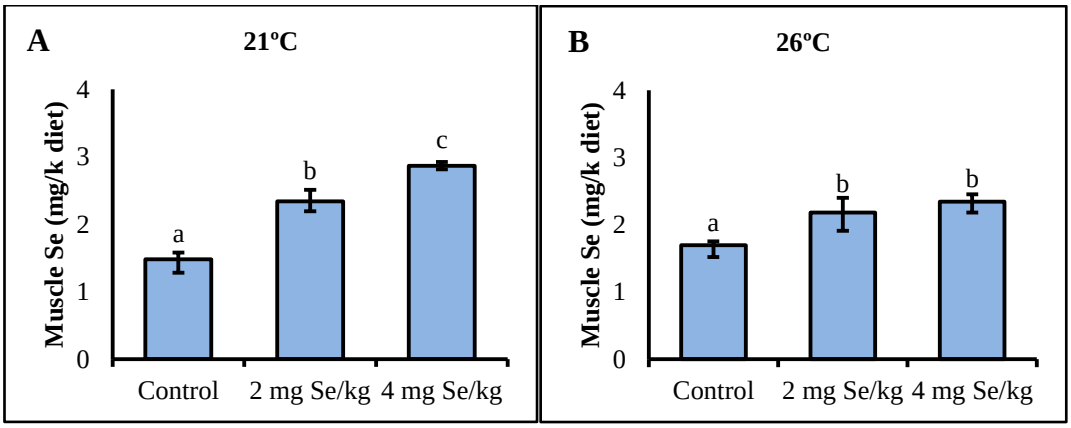

Figure 3.2. Selenium concentration of muscles of juvenile yellowtail kingfish reared at 21°C (A) and 26°C (B) water temperatures.

The histological analysis of the yellowtail kingfish showed necrotic fibres in the muscles of juvenile yellowtail kingfish fed with the control diet (Figure 3.3). A histopathological test confirmed that 20.3% of fish exhibiting lesions in skeletal muscles occurred in the absence of Se supplementation in the fish diet. However, the histological alteration in the muscles of yellowtail kingfish that were fed the control diet, from both ambient and elevated water temperatures, was similar. Additionally, an insignificant amount of fish (6 from 26°C) exhibited eye problems, which were detected during the final sampling.

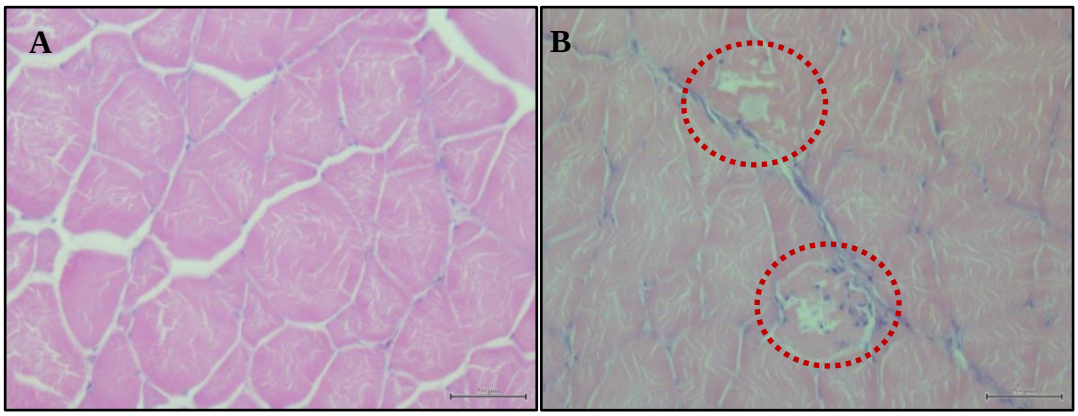

Figure 3.3. Histopathological analysis on juvenile yellowtail kingfish muscles showing the normal (A) and necrotic changes (B), scale bar = 50 μm.

3.4. Discussion

Both water temperature and Se level interactively modified the growth performance (FW and SGR) of yellowtail kingfish, with the overall final weight and SGR were highest at 21°C (128.7 g and 2.2% body weight day^{-1}). The interaction between temperature and Se exposure observed in this study, suggested that elevated temperature was a strong inducer of low FW and reduced growth in yellowtail kingfish. It appeared that, for Se, elevated temperature exacerbated toxicity. As water temperature increased, the fish fed diets supplemented with 2.0 mg kg^{-1} decreased their FW and SGR by about 30% and 54%, respectively. The imbalance between respiratory demands and respiratory capacity might have caused a physiological stress that resulted in lower FW and SGR of fish in the elevated temperature. Moreover, at 26°C, the energetic demands of increased oxygen consumption and reduced respiratory capacity were not counteracted by an increase in feeding activity, indicating that Se could have placed further metabolic stress on these fish.

However, of the limited information available on the synergistic effects of Se and temperature on aquatic animal growth and health indicators, poor reproduction has been detected when Se-deficient planktonic crustacean (*Daphnia magna*) were kept at 25°C, whilst such an effect was not detected at 20°C (Winner & Whitford, 1987). The so-called "winter stress syndrome" of Lemly (Lemly, 1996 ; Lemly, 1993), signalled by increased mortality, reduced activity and feeding as well as the reduced condition factor and decreased energy stores of bluegill sunfish (*Lepomis macrochirus*), is the upshot of winter temperatures combined with elevated Se exposure. In addition, there is also a synergism between Se and raised temperatures causing mortalities and abnormal morphologies in white sturgeon (*Acipenser transmontanus*) and green sturgeon (*A. medirostris*) (Silvestre et al., 2010).

It appears from this study that, irrespective of the diet, rearing yellowtail kingfish at the elevated temperature of 26°C significantly resulted in reduced growth performance. The present finding contrasts with that of Abbink et al. (2012) which found that increasing the water temperature from 21°C to 26.5°C resulted in a 54% increase in the FW, after one month's exposure. Aside from the differences in culture conditions, this contradiction could be attributed to fish size. The larger IW (67g) of yellowtail kingfish in the present study than used by Abbink et al. (2012) could result in an ontogenetic shift in optimum temperature for growth as shown in majority of ectothermic animals (Angilletta & Dunham, 2003) such as cod (*Gadus morhua* L.) (Björnsson et al., 2001) and turbot (*Scophthalmus maximus* L.) (Imsland et al., 1996).

It has been shown that the temperature required for optimum growth declines by 1–2°C when the larger fish (10 – 250 g) were used (Cuenco et al., 1985). Similar to the finding of the present study, Pirozzi and Booth (2009) examined the influence of temperature exposure ranging from 10 to 35°C on the oxygen consumption or routine metabolic rate (RMR) of naturally occurring yellowtail kingfish and found that the thermo-sensitivity reaction of RMR appears as an indicator of the temperature profile and suggesting the preferred temperature ranges from 20 to 25°C. Martin and Huey (2008), through their "optimality model", predicted that animals, if given a choice, will select temperatures that are somewhat lower than the temperature at which fitness is optimum. Therefore, the growth retardation at 26°C,

as observed in this study, can be due to the increased metabolic rate at an elevated temperature. However, the increased demand for energy by yellowtail kingfish was not correspondingly denoted by an increase in relative feed intake (RFI), a circumstance that is normally observed when fish are exposed to temperature stress (Handeland et al., 2008).

In fish, thermal stress is accompanied by oxidative stress (Lushchak, 2011). Enzymatic antioxidant GPx plays a vital role in maintaining cellular defence against extreme ROS production and lipid peroxidation (Kohen & Nyska, 2002 ; Pedrero & Madrid, 2009). GPx metabolises hydroperoxides and is thus intimately engaged in cellular defence against oxidative damage (Arthur et al., 2003). Therefore, Se is an essential micro-nutrient of major metabolic importance (Brown & Arthur, 2001 ; Hamilton, 2004). The results of the present study show a significant increase in the RBC GPx activity in elevated water temperatures, at least at 26°C and the experimental conditions employed in the present study. This indication reinforces the proposed hypothesis that the dietary requirement of Se is relatively higher in fish, in response to oxidative stress induced by an increased temperature level.

Although there was no statistical difference in erythrocyte GPx activity between the Se supplementation levels of 2.0 and 4.0 mg kg^{-1} diet at both 21°C and 26°C temperature regimes, enzymatic GPx activity levels were significantly higher in the erythrocytes of fish with Se supplementation than in those without Se supplementation. This would suggest that the dietary Se supplementation of 2.0 mg kg^{-1} in the commercial FM-based diets is needed to maintain maximum RBC GPx activity. Again, apparent enzymatic GPx activity levels, as observed in this study, confirm the earlier suggestion with similar species that Se supplementation has been reported to promote the enhanced physiological response of GPx activity during stress periods (Le & Fotedar, 2014b).

The significance of Se to the antioxidant capacity in other species has also been documented (Chiu et al., 2010 ; Lin & Shiau, 2005 ; Liu et al., 2010 ; Rider et al., 2009 ; Wang et al., 2006a ; Zhou et al., 2009). Furthermore, Se-containing compound, selenoneine, was found to be the major form of organic Se in the muscle and other tissues of tuna, mackerel and tilapia (Yamashita et al., 2010). Se may exert an antioxidant effect by binding to oxygen-binding proteins such as haemoglobin

(Hb) and myoglobin, protecting them from autooxidation (Yamashita & Yamashita, 2015). The implication of Se for the growth performance of yellowtail kingfish might be associated with the antioxidant role of Se.

Se plays a vital role in amino acid metabolism and is also incorporated into some proteins. Organically bound Se, such as Se-met and Se-cys, is efficiently absorbed throughout the gastrointestinal tract (Gropper et al., 2009). Although Se-cys is a more available source of Se for selenoprotein synthesis, only Se-Met is incorporated into body proteins (Schrauzer, 2000). Therefore, Se accumulation should occur in protein-rich tissues such as skeletal muscle (Dainty & Fox, 2005). In fish, it has been proved that muscle tissue is one of the primary sites of Se storage (Burk & Hill, 1993). In the present study, supplementing yellowtail kingfish diets with organic Se from a commercial product, in the form of Se-met (Sel-Plex®), led to a considerable increase in the concentration of Se in muscles. The overall increased accumulation of muscle Se in yellowtail kingfish that were fed Se-enriched diets, regardless of water temperature, was likely to have been caused by the non-specific incorporation of Se-met into proteins.

Tissue accumulation of Se may be understood, with respect to bioavailability, as a sign of the superiority of organic sources over inorganic sources (Ornsrud & Lorentzen, 2002). In fact, at 21°C, there was a linear relationship between organic Se supplementation levels and Se accumulation in muscles. This finding parallels those reported for other fish. For example, a linear response was observed in channel catfish (*I. punctatus*) with respect to the dose in Se accumulation in muscles following 15 weeks of an organic Se (ranging from 0 to 15 mg Se kg^{-1}) feeding trial (Gatlin & Wilson, 1984). Similarly, during eight weeks of exposure, accumulation in the muscle Se of Atlantic salmon (*Salmo salar*) parr was directly related to dietary Se, when organic Se was included in the diet (Lorentzen et al., 1994). In contrast, at 26°C, Se concentration in the muscles of yellowtail kingfish supplemented with a 2.0 mg Se kg^{-1} diet, was not significantly different from that supplemented with a 4.0 mg Se kg^{-1} diet, and these concentrations were lower, compared to those at 21°C. The reduction in muscle Se concentrations, as noticed in the present study, might be ascribed to the increased utilisation of Se against extreme thermal induced ROS production.

Elevated temperatures may also trigger osmotic imbalance, as shown in the present study. Serum osmolality was greater in fish kept at 26°C (>305 mOsm kg^{-1}) than in those kept at 21°C. Although the osmotic pressure of the aqueous humour (watery fluid similar to plasma located in the anterior and posterior chambers of the fish eye) was not measured in the present study, there was a linearity between serum osmolality and aqueous humour osmolality, being indicative of cataract formation in salmonids (Iwata et al., 1987). Akiyama et al. (1986) studied the effects of temperature on the incidence of scoliosis and cataracts in chum salmon (*Oncorhynchus keta*) fry, and found that cataracts are apt to occur when metabolism is high, due to increased water temperatures. Similarly, Atlantic salmon (*S. salar*) parr kept at a constant higher temperature, developed more cataracts than parr raised at a constant, low water temperature (Bjerkås et al., 2001). Despite the insignificant number of fish that were found to exhibit a somewhat cloudy eye during the final sampling, it is not clear whether the signs of eye impairment are a direct or secondary effect of osmotic imbalance. Hence, further study should investigate whether acute or chronic Se exposure plays a part in the formation of the so-called "cloudy eye syndrome" or cataracts, one of the common disorders of the eye in finfish (Hargis Jr, 1991).

In aquatic wildlife, Se is a widespread and naturally occurring element (US-EPA, 2004). Bioaccumulation of Se through food, leading aquatic animals to high levels of Se exposure, has been a significant concern. Thus, at levels exceeding those required, Se can induce harmful effects, which may be described principally by the failure of the protein metabolism to distinguish sulfur (S) amino acids and their seleno analogues. Because it is most similar to S through its chemical properties, Se is faultily substituted for S, when generated in excessive amounts (Meyer et al., 1992). This may result in the formation of triselenium linkage (Se–Se–Se) or selenotrisulfide linkage (S–Se–S), either of which impede the formation of the functionally essential disulfide chemical bonds (S–S) (Lemly, 2002). The substitution of Se for S eventually results in weakened, dysfunctional enzymes and protein structures, which damages normal cellular biochemistry (Lemly, 2002).

Those Se-induced flaws in the protein biosynthesis bring about several metabolic consequences. Symptoms of Se toxicity in fish include elevated mortalities, lower

feed intake, histopathological alterations in tissues, poor reproductive performance, and decreased growth response and haematocrit values (Gatlin & Wilson, 1984 ; Hamilton et al., 2002 ; Hilton et al., 1980 ; Jaramillo Jr et al., 2009 ; Lemly, 1997 ; Sorensen et al., 1984 ; Tashjian et al., 2006). However, the toxicity of Se appears to be impacted by the length of exposure and life stages of the affected animal (Lemly, 2002). The earliest life stages of fish are more Se-sensitive (Lemly, 1997), suggesting that those at embryo-alevin-fry stages may pose a higher risk of elevated Se levels than those at later stages, such as juvenile and adult fish. Whilst the diet containing 15.43 mg Se kg^{-1} did not induce any toxic effects in 19.55 g yellowtail kingfish after 10 weeks of exposure (Le & Fotedar, 2014c), the diet with 13 mg Se kg^{-1} caused reduced growth, poor feed intake, and high mortality in 1.3 g rainbow trout (*O. mykiss*), after four weeks of feeding (Hilton et al., 1980). In spite of the proposed threshold level between 15.43 and 20.87 mg Se kg^{-1} for yellowtail kingfish (Le & Fotedar, 2014c), the absence of detrimental effects on the growth and health parameters of juvenile yellowtail kingfish fed the highest Se supplementation level (4.0 mg kg^{-1}, providing the actual concentration of 7.38 mg kg^{-1}), employed in the present study, can therefore be established.

Conversely, Se deficiency in fish may manifest as the malfunction of various organs and tissues, including skeletal muscle, as described by Chariot and Bignani (2003). Muscle fibre degeneration necrosis of sea bass (*Dicentrarchus labrax*) has been associated with Se inadequacy in diets (López-Albors et al., 1995). Correspondingly, in the present study, in addition to the low antioxidant capacity of GPx, fish that were fed the control diet, exhibited muscle lesions, which is characterised by an alteration of skeletal muscle fibres, initiating contraction impairment, muscle atrophy and various degrees of limb or trunk severity (Lescure et al., 2009). Similar muscle conditions have been reported in various fishes, such as rainbow trout (*O. mykiss*) and Atlantic salmon (*S. salar*) (Bell et al., 1986 ; Lorentzen et al., 1994). White muscle disease in domestic animals is also caused by a deficiency in Se, which negatively affected productive efficiency and animal health (Hefnawy & Tórtora-Pérez, 2010). In the present study, this "nutritional muscular dystrophy syndrome" occurred because the biological availability of Se from FM appeared to be low, possibly due to the binding of Se to mercury and other heavy metals (Webster & Lim, 2002). Therefore, Se supplementation may be required, to avoid deficiency

syndromes, as well as maintain optimal growth and a functional immune system, in yellowtail kingfish.

3.5. Summary

In summary, the present study has demonstrated that there is an interactive relationship between Se level and temperature, in yellowtail kingfish. Se-supplemented diets significantly increased the final weight and SGR of fish reared at 21°C, but not at 26°C. Reduced growth, lower Se concentration in muscles and higher osmolality of fish reared at elevated water temperature might be linked to thermal-induced oxidative stress. However, the antioxidant capacity of GPx, muscle Se level, and muscle histological performance were influenced by dietary Se intake. The present outcome may be relevant in portraying the impacts of temperature and Se level on antioxidant capacity, muscle histochemistry and growth for other marine finfish species.

CHAPTER 4: Use of organic selenium supplements in soybean meal-based diets for juvenile yellowtail kingfish (*Seriola lalandi*)

This chapter has been published in International Journal of Food and Nutritional Science
Volume 3 Issue 2 (2016): 1-11

4.1. Introduction

One of the major challenges facing the aquaculture sector worldwide is the limited utility of formulated feed containing fishmeal (FM) from wild sources. Soybean meal (SBM) is among the most attractive plant protein (PP) substitutes for FM (Day & Howell, 1997) due to its satisfactory protein and amino acid content, wide availability, and reasonable price (Venou et al., 2006). The partial or full substitution of SBM for FM in finfish diets has been studied over the past two decades (Collins et al., 2012 ; Gallagher, 1994 ; Glencross et al., 2005 ; Hernández et al., 2007 ; Kokou et al., 2012 ; Olsen et al., 2007 ; Silva-Carrillo et al., 2012 ; Suarez et al., 2013 ; Tibaldi et al., 2006 ; Vielma et al., 2000). These studies have revealed that various levels of SBM are acceptable for inclusion in place of FM in terms of both the nutrient composition of SBM diets and performances of the fish studied.

However, one major drawback to the utilisation of PP ingredients in this context is the presence of certain antinutritional factors (ANFs): innate substances that can impair utilisation and digestion of nutrients (Francis et al., 2001). Phytic acid is one of the most common ANFs in PP feedstuffs, since around 56–81% of total phosphorus (P) in SBM is stored as phytate (Selle et al., 2003). This phytic acid-bound P cannot be hydrolysed by monogastric animals, including fish (Refstie & Storebakken, 2001). Moreover, phytate chelates divalent mineral cations such as iron (Fe), zinc (Zn), magnesium (Mg), manganese (Mn), copper (Cu), calcium (Ca) and selenium (Se), thus limiting availability of P and other minerals in SBM-based diets (Francis et al., 2001 ; Harland & Morris, 1995). Phytate may also form insoluble complexes with protein and amino acid (AA) cations in PP feedstuffs, diminishing their digestibility for fish (Kumar et al., 2012). Consequently, supplementation of trace minerals has been applied to the diets of several fish species to improve mineral

bioavailability (Paripatananont & Lovell, 1998 ; Satoh, 2007 ; Watanabe et al., 1997).

Se supplementation has been reported to beneficially affect the performance of various fish species. Although the element is only required in trace amounts, Se is an essential nutrient for aquatic organisms due to being an integral component of selenocysteine (Se-cys)-containing proteins, which are involved in most aspects of cell biochemistry and functioning (Arthur et al., 2003). Moreover, Se is employed in the antioxidant enzyme glutathione peroxidase (GPx) (Barciela et al., 2008 ; McKenzie et al., 2002 ; Thiry et al., 2012), which, in fish, may provide protection against oxidative stress following exposure to physical and environmental stressors (Rider et al., 2009). Se is also associated with protein in the muscle tissues of marine animals (Maher, 1985). Fish may absorb minerals directly from the surrounding water, but diet is their major source of Se (Lall, 2003). The varying Se requirements and toxicity levels for fish on a FM-based diet have been documented (Abdel-Tawwab et al., 2007 ; Elia et al., 2011 ; Gatlin & Wilson, 1984 ; Hilton et al., 1980 ; Lin & Shiau, 2005 ; Pacini et al., 2012); these differences may depend on the concentration and type of compound absorbed, the duration and mode of exposure, and fish species in question.

However, research is still required into the nutritional implications of Se-deficient, PP-based diet on fish growth and physiology, as this could be used to inform further dietary modifications for cultured fish. This work is of particular interest in relation to emerging aquaculture species, such as yellowtail kingfish (*Seriola lalandi*). Although several studies have investigated yellowtail kingfish nutrition (Booth et al., 2013 ; Bowyer et al., 2013a ; Buentello et al., 2015 ; Le & Fotedar, 2013b ; Le et al., 2014b), no previous work has examined the relationship between Se-enriched SBM diets and the growth and health of the species. Yellowtail kingfish may, due to the presence of certain ANFs, have a relatively high requirement for the antioxidant properties of Se. Therefore, the present study was designed to determine the growth and physiological performance of yellowtail kingfish when fed Se-supplemented, SBM-based diets. The study achieved this goal by exposing juvenile yellowtail kingfish to low and high levels of dietary SBM inclusion, with and without Se

supplementation, for 60 days and then evaluating survival and growth performance, GPx activity and muscle histopathology.

4.2. Materials and methods

4.2.1. Experimental diets

The formulation and proximate content of the diets are shown in Table 4.1. All ingredients were ground to pass through a 1-mm mesh screen, pelleted in a mixer, crumbled to the desired size, air-dried, and stored at 4°C until feeding. Five diets were formulated: 0% SBM (SBM_0, control), low SBM (25%) without and with organic selenium (OS) supplements (SBM_{25}; SBM_{25+Se}), and high SBM (75%) without and with OS supplements (SBM_{75}; SBM_{75+Se}). The control diet (SBM_0) was supplemented with OS to meet the Se requirement of yellowtail kingfish. All experimental diets were prepared to be isonitrogenous (49% crude protein) and isoenergetic (22 MJ kg^{-1} gross energy) basis. Chromic oxide (Cr_2O_3) was included in all diets at 0.5% as an inert, indigestible marker. The Se dosage for dietary supplementation was based on our preliminary study and similar to the recommendation of Le and Fotedar (2013a) for the optimal growth of yellowtail kingfish. Amino acid (AA) profiles of the experimental diets are shown in Table 4.2.

Table 4.1. Formulation and composition of the experimental diets

Ingredients[a] (g kg^{-1} dry weight)	SBM_0	SBM_{25}	SBM_{25+Se}	SBM_{75}	SBM_{75+Se}
FM	460	340	340	180	180
Soybean meal[b]	-	150	150	430	430
Wheat gluten	100	100	100	100	100
Wheat flour	80	30	30	10	10
Casein	120	120	120	120	120
Fish oil	100	110	110	110	110
Wheat starch	65	50	50	25	22
Cellulose	50	75	72	-	-
Se-free premix[c]	20	20	20	20	20
Sel-Plex	2.5	-	3	-	4
Chromic oxide	5	5	5	5	5
Proximate content (%)					
Dry matter	85.17	88.65	89.21	90.38	89.72
Ash	8.25	7.86	7.73	7.43	7.22
Protein	49.28	49.08	49.15	49.30	49.16
Lipid	15.06	14.85	14.86	14.90	14.66
Gross energy (MJ/kg)	21.33	22.16	22.10	22.13	22.07
Se (mg kg^{-1})	5.63	2.56	5.53	1.52	5.51

[a] Supplied by Specialty Feeds, Perth, WA, Australia, except for Sel-Plex and chromic oxide, obtained from AllTech, Lexington, Kentucky, USA and Thermo Fisher Scientific, Scoresby, Vic, Australia, respectively.

[b] Solvent-extracted; Malaysian origin

[c] Contains the following (as g kg^{-1} of premix): iron, 10; copper, 1.5; iodine, 0.15; manganese, 9.5; zinc, 25; vitamin A retinol, 100 IU; vitamin D3, 100 IU; vitamin E, 6.25; vitamin K, 1.6; vitamin B1, 1; vitamin B2, 2.5; niacin, 20; vitamin B6, 1.5; calcium, 5.5; biotin, 0.1; folic acid, 0.4; inositol, 60; vitamin B12, 0.002; choline, 150; ethoxyquin, 0.125.

Table 4.2. Hydrolysed amino acid composition of FM, SBM, wheat gluten and casein (g 100 g^{-1} protein)

Amino acid	FM	SBM	Wheat gluten	Casein
Essential				
Arginine	4.35	3.69	1.50	3.32
Histidine	2.09	1.23	0.96	2.73
Isoleucine	3.04	2.25	2.35	4.70
Leucine	5.17	3.88	9.75	8.70
Lysine	4.77	2.83	0.80	7.46
Methionine	1.86	0.58	1.05	2.19
Phenylalanine	2.87	2.61	3.47	4.67
Threonine	3.19	1.89	1.76	3.71
Valine	3.26	2.14	2.58	5.75
Non-essential				
Alanine	4.42	2.04	NA	2.56
Aspartic acid	6.20	5.48	NA	6.24
Glutamic acid	8.25	8.96	NA	18.60
Glycine	4.73	1.94	NA	2.38
Proline	3.81	3.01	NA	8.80
Serine	3.05	2.64	NA	5.36

NA: not analysed

4.2.2. Fish and experimental design

Juvenile yellowtail kingfish (mean weight 2.95 g) were obtained from the Australian Centre for Applied Aquaculture Research, Fremantle, Western Australia (WA), Australia. Fish were fed with a commercial diet (Gemma Diamond 1.5, Skretting, France) for one week until fully acclimated to the rearing conditions. Fish were then randomly sorted into 15 experimental tanks, with three tanks of 15 fish each for each diet. Fish handling procedures, care standards, and facilities all complied with the guidelines of the Animal Ethics Committee of Curtin University and followed the Australian Code of Practice for the care and use of animals for scientific purposes.

The experimental system consisted of 15 circular, 300-L fibreglass tanks installed at the Curtin Aquatic Research Laboratory (CARL), Curtin University, Australia. Each

tank received recirculated water from an external biofilter (Fluval 406, Hagen, Italy) at 10 L min^{-1}. 50% of the water was exchanged twice weekly. All experimental tanks were supplied with constant aeration and pure oxygen (compressed oxygen, BOC, Perth, WA, Australia).

Throughout the experiment, the water quality parameters were monitored daily. Dissolved oxygen (DO) was monitored using a DO meter (CyberScan DO 300, Eutech Instruments, Singapore). Ammonia and pH were measured every two days using chemical test kits (Aquarium Pharmaceuticals Ltd, UK) and a pH meter (CyberScan pH 300, Eutech Instruments, Singapore) respectively. Water salinity was measured using a portable refractometer (RHS-10ATC). During the experiment, water temperature, pH, dissolved oxygen, and salinity were maintained at (mean ± SD) 21.2 ± 0.3 °C, 7.5 ± 0.1, 6.0 ± 0.3 mg l^{-1}, and 34.2 ± 0.3 ppt, respectively. The photoperiod consisted of 12 hours of fluorescent light per day.

4.2.3. Digestibility measurement

Fish were hand-fed to satiation twice a day, at 09:00 and 15:00 hours. To investigate the effect of dietary treatment on digestibility, one week before the end of the feeding experiment faecal matter was collected immediately prior to the morning feeding using stripping techniques (Austreng, 1978). Faecal collections from individuals were pooled by tank and quickly stored at –20ºC. Prior to analysis, the faecal samples were dried to constant weight at 105 ºC. Apparent digestibility coefficients (ADCs) were measured using the indirect method (Cr_2O_3) as suggested by Cho et al. (1982).

4.2.4. Sampling and analytical methods

Fish weight sampling was conducted at the beginning of the feeding trial and at 15-day intervals thereafter. At the end of the feeding trial, all fish were starved for 24 hours prior to final sampling to achieve a basic metabolite state. The fish were then anaesthetised with tricaine methanesulfonate (MS-222, Sigma-Aldrich, Castle Hill, NSW, Australia) at 100 mg L^{-1} before sampling.

Fish blood samples were drawn by caudal vein puncture with a 1-mL plastic syringe. The extracted blood was then transferred to K_2EDTA-containing tubes (BD

Vacutainer® Plus Plastic) for haematology. An Hb kit (Randox Laboratories, Antrim, United Kingdom) was used to measure the haemoglobin (Hb) content. Erythrocyte (red blood cell) glutathione peroxidase (GPx) activity was quantitatively assayed using the Randox Laboratories combined test (Ransel, Antrim, United Kingdom). Enzyme activity is expressed in U g Hb^{-1}.

The proximal compositions of the diet and faecal samples were determined based on Association of Official Analytical Chemists procedures (AOAC, 1990). Dry matter was determined by oven drying to constant weight at 105ºC; crude ash by combustion at 550 ºC; crude protein content (N × 6.25) by the Kjeldahl digestion method; crude lipid content by the Soxhlet technique; and gross energy content by an IKA oxygen bomb calorimeter (Heitersheim, Germany).

4.2.5. Selenium assay

Se in the muscle tissue and experimental diets was analysed at the Intertek Genalysis Laboratory (Perth, Australia) using inductively coupled plasma atomic emission spectrometry (ICP-AES).

4.2.6. Histopathology assay

At the end of the trial, tissue samples from five fish from each tank were collected, fixed in 4% saline and formalin, then dehydrated in ethanol before equilibration in xylene and embedded in paraffin wax. Sections of approximately five µm were cut and stained with haematoxylin and eosin for histological examination under an Olympus BX40F4 light microscope. Light microscopy samples were prepared according to standard histological techniques (Luna, 1968).

4.2.7. Calculations

Growth and feeding performances were measured using the calculated parameters below:

$$\text{SGR (\% day}^{-1}) = 100 \times \left[\frac{\ln \text{FW} - \ln \text{IW}}{n \text{ days}}\right]$$

$$\text{WG (\%)} = 100 \times \left[\frac{\text{FW-IW}}{\text{IW}}\right]$$

$$\text{FI (g fish}^{-1}\text{ days}^{-1}) = \left[\frac{\text{diet given} - \text{diet remaining}}{\text{number of fish}}\right] / \text{days}$$

$$FCR = \frac{FI}{WG}$$

$$S\ (\%) = 100 \times \left[\frac{\text{final number of fish}}{\text{initial number of fish}}\right]$$

$$ADC_{Nutrient}\ (\%) = 100 \times \left[\left(1 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diet}}\right)\right]$$

$$ADC_{Dry\ matter}\ (\%) = 100 \times (1 - \%\ Cr_2O_3 \text{ in diet} \,/\, \%\ Cr_2O_3 \text{ in faeces})$$

Where: SGR (specific growth rate); FW (final weight); IW (initial weight); WG (weight gain); FI (feed intake); FCR (feed conversion ratio); S (survival); ADC (apparent digestibility coefficient).

4.2.8. Statistical analysis

The results were analysed using one-way analysis of variance (ANOVA, IBM SPSS Statistics, Australia). When appropriate, a post-hoc test (utilising the Duncan method) was applied to examine differences among the treatment groups, with a probability value of $P < 0.05$ indicating significant differences. The relationship between Se concentration and fillet Se content was evaluated using regression analysis. All data are expressed as means of three replicates ± standard error (SE). Data expressed as percentages were arcsine transformed prior to statistical analysis.

4.3. Results

4.3.1. Growth and feed utilisation

Although the FI values of fish fed high SBM diets were lower, they did not differ significantly from those fed on the control or low SBM diets, ranging from 1.36 to 1.52 g fish^{-1} day^{-1}. All experimental diets were accepted by the fish throughout the course of the feeding trial. Figures for fish growth performance, survival, FI and FCR are presented in Table 4.3. The FW of fish fed low SBM diets did not differ significantly from that of fish fed the control diet ($P > 0.05$), though FW was significantly reduced when fish were fed high SBM diets ($P < 0.05$). However, OS supplementation improved FW for both low and high concentrations of SBM protein in diets. Although OS supplementation had no effect on SGR for low SBM diets, it did when fish were fed high SBM diets and the SGR of fish fed the SBM_{75+Se} diet was significantly higher than that of fish fed the SBM_{25} diet.

In addition, an improved FCR was observed in fish fed with OS-supplemented diets, although no difference in SGR was found between the SBM_{75} and SBM_{75+Se} dietary groups. The changes in the mean body weight of juvenile yellowtail kingfish during the experimental period are presented in Figure 4.1. All groups of fish had similar body weights during the first two weeks of the trial, after which growth deterioration began in fish fed high SBM diets. High survival rates, exceeding 95%, were found for all dietary treatments during the experiment.

Table 4.3. Growth performance, survival, FI, and FCR in juvenile yellowtail kingfish fed the test diets for 60 days

Indicators	Diet				
	SBM_0	SBM_{25}	SBM_{25+Se}	SBM_{75}	SBM_{75+Se}
IW	4.93 ± 0.07	5.03 ± 0.11	5.01 ± 0.06	5.02 ± 0.08	5.12 ± 0.06
FW	81.08 ± 1.10^{ab}	79.64 ± 1.55^{bc}	85.20 ± 1.52^{a}	67.89 ± 1.31^{d}	75.64 ± 1.27^{c}
SGR	4.67 ± 0.03^{a}	4.60 ± 0.03^{ab}	4.73 ± 0.04^{a}	3.37 ± 0.06^{c}	4.49 ± 0.05^{b}
S	98.33 ± 1.67	96.7 ± 3.30	96.7 ± 3.30	95.0 ± 2.88	98.3 ± 1.67
FI	1.42 ± 0.09	1.52 ± 0.02	1.47 ± 0.03	1.36 ± 0.02	1.38 ± 0.06
FCR	1.20 ± 0.02^{b}	1.22 ± 0.01^{b}	1.10 ± 0.01^{a}	1.24 ± 0.03^{b}	1.17 ± 0.07^{ab}

IW: initial weight (g); FW: final weight (g); SGR: specific growth rate (% body weight day^{-1}); S: survival (%); FI: feed intake (g $fish^{-1}$ day^{-1}); FCR: feed conversion ratio (g fed g $gained^{-1}$)
Data are expressed as means ± SE. Means in the same row with different superscript letters are significantly different ($P < 0.05$).

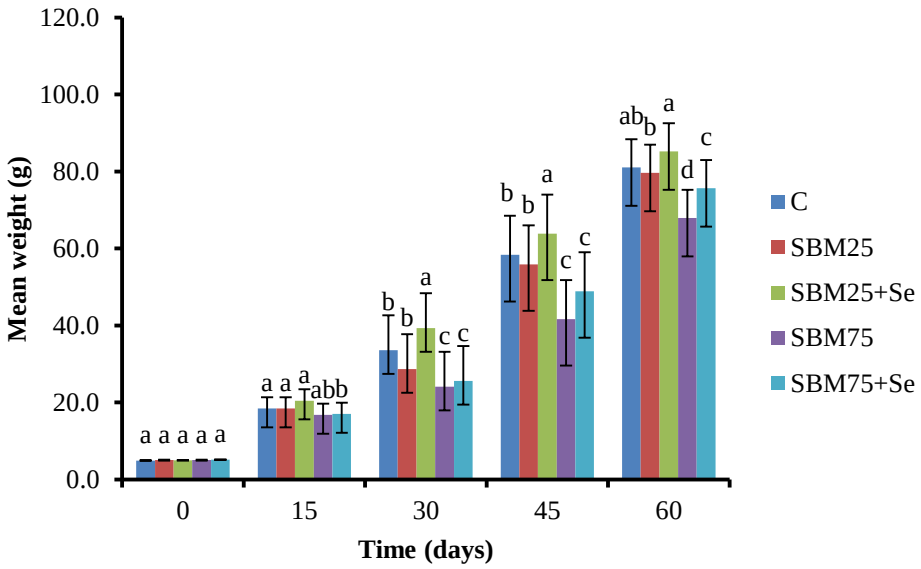

Figure 4.1. Changes in the mean body weight of juvenile yellowtail kingfish fed different diets during the experimental period.

4.3.2. Nutrient composition

The proximate composition, gross energy and Se concentration of fillets from yellowtail kingfish fed experimental diets are displayed in Table 4.4. At the end of the feeding trial, crude lipid, ash, moisture and gross energy content did not differ significantly between dietary treatments ($P > 0.05$). However, crude protein in fillet was significantly reduced with the increase of SBM as a proportion of the diets ($P < 0.05$), while fish fed diets supplemented with OS accumulated more Se in their fillets than those fed on other diets ($P < 0.05$). A linear relationship was observed between fillet Se content and dietary Se supplementation (Figure 4.2).

Table 4.4. Proximate composition, gross energy, and Se concentration of fillets from yellowtail kingfish fed experimental diets

Proximate composition	Diet				
	SBM_0	SBM_{25}	SBM_{25+Se}	SBM_{75}	SBM_{75+Se}
Protein (%)	21.2 ± 0.40^{a}	21.4 ± 0.61^{a}	21.3 ± 0.64^{a}	19.4 ± 0.48^{b}	19.5 ± 0.34^{b}
Lipid (%)	2.23 ± 0.11	2.16 ± 0.21	2.18 ± 0.89	2.19 ± 0.73	2.24 ± 0.16
Ash (%)	1.28 ± 0.30	1.41 ± 0.50	1.38 ± 0.60	1.29 ± 0.20	1.33 ± 0.30
Moisture (%)	73.6 ± 1.32	72.7 ± 1.47	72.8 ± 1.81	73.3 ± 0.72	72.4 ± 1.43
Gross energy (MJ kg^{-1})	5.17 ± 0.31	5.08 ± 0.16	5.11 ± 0.10	4.93 ± 0.34	5.05 ± 0.17
Se (mg kg^{-1})	0.41 ± 0.06^{b}	0.38 ± 0.09^{b}	0.71 ± 0.04^{a}	0.32 ± 0.04^{c}	0.55 ± 0.06^{a}

Data are expressed as means ± SE. Means in the same row with different superscript letters are significantly different ($P < 0.05$).

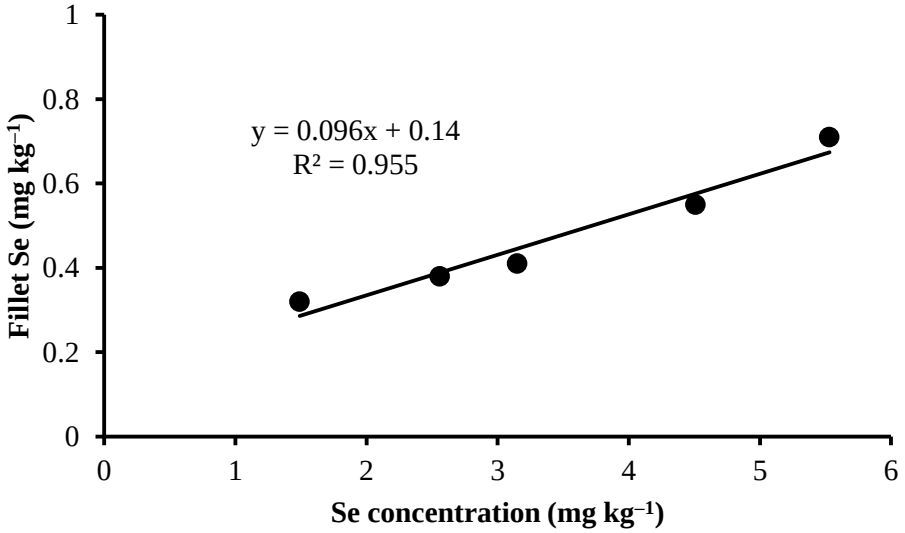

Figure 4.2. Relationship between fillet Se content and dietary Se concentration of juvenile yellowtail kingfish after 60 days.

4.3.3. Apparent digestibility coefficient (ADC)

Table 4.5 presents the data for apparent digestibility coefficient (ADC) of protein, lipid and dry matter. ADC of protein and dry matter was not affected by OS supplementation, but was influenced by SBM levels. Furthermore, while dietary replacement of FM protein with SBM at moderate (25%) or substantial (75%) levels resulted in insignificant ADC scores for lipids, the ADC of proteins and dry matter was significantly reduced as the proportion of SBM in the diets increased.

Table 4.5. Apparent digestibility coefficients (ADC) of the nutrients in the five experimental diets

ADC (%)	Diet				
	SBM_0	SBM_{25}	SBM_{25+Se}	SBM_{75}	SBM_{75+Se}
Protein	94.87 ± 1.24^{a}	90.42 ± 1.41^{b}	90.75 ± 0.92^{b}	85.62 ± 1.63^{c}	85.87 ± 1.57^{c}
Lipid	96.75 ± 0.97	95.79 ± 1.50	95.51 ± 1.26	96.63 ± 0.94	96.25 ± 1.13
Dry matter	90.25 ± 1.63^{a}	88.85 ± 0.83^{a}	88.56 ± 1.14^{a}	82.73 ± 1.66^{b}	82.13 ± 1.35^{b}

Data are expressed as means ± SE. Means in the same row with different superscript letters are significantly different ($P < 0.05$).

4.3.4. Glutathione peroxidase (GPx) activity

The GPx activity in the red blood cells (RBC) of yellowtail kingfish for each dietary treatment is summarised in Figure 4.3. GPx activity tended to decrease with decreasing levels of Se in the diets. The fish fed SBM_{25+Se} had a higher GPx activity ($P < 0.05$) than with those fed other diets. The lowest GPx activity was observed in fish fed the SBM_{75} diets.

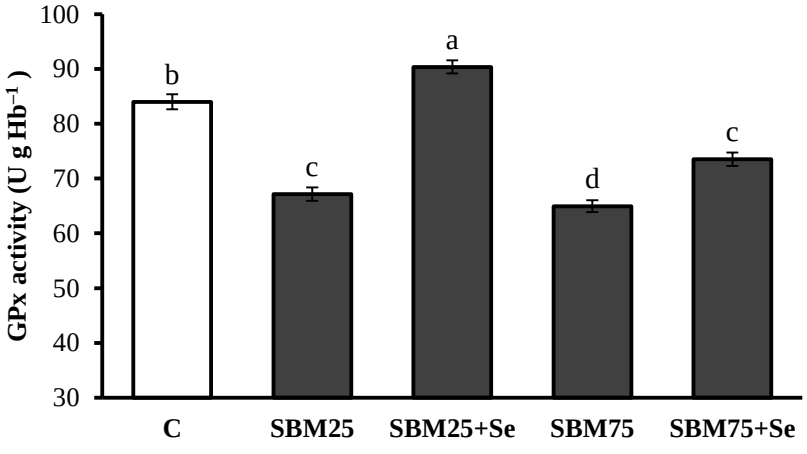

Figure 4.3. Glutathione peroxidase (GPx) activity in the red blood cells of juvenile yellowtail kingfish fed experimental diets.

4.3.5. Histopathological observation

The histological profiles of the fish tissue samples from the control, SBM_{25}, SBM_{25+Se}, SBM_{75}, and SBM_{75+Se} groups are shown in Figure 4.4. Tissue samples from fish fed the different dietary treatments were compared to those from fish fed the control diet, with several histopathological alterations evident in those fed diets without OS supplementation. These pathological findings included the deterioration of muscle bundles, with the aggregation of inflammatory cells between bundles and necrotic changes. The alterations were most prevalent in fish fed the SBM_{75} diet, with four of five samples exhibiting such changes.

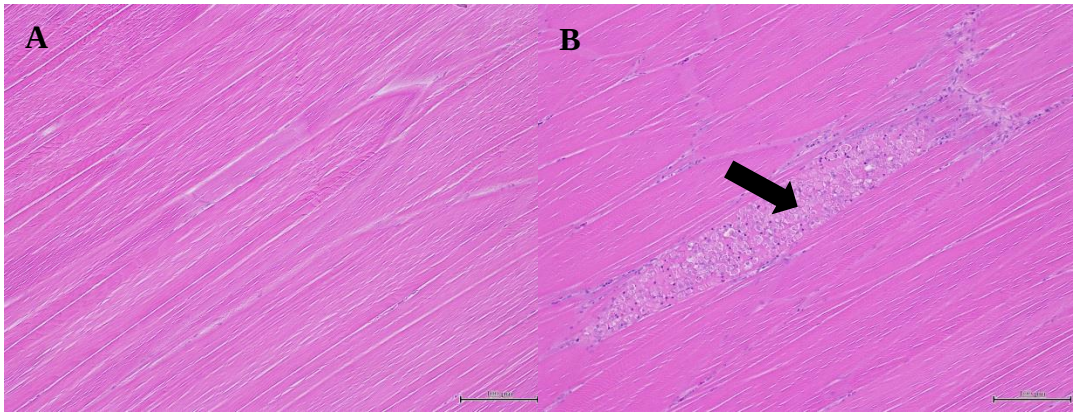

Figure 4.4. Histological examination of yellowtail kingfish. The tissues of fish fed the control and OS-supplemented diets exhibit a normal shape (A), while Se-induced myopathy (marked with the arrow) is observed in the tissues of fish fed with SBM diets without OS supplement (B) (scale bar = 100 μm).

4.4. Discussion

Following the recognition of Se as an essential element in aquaculture feeds, numerous formulations have been supplemented with this chemical to enhance diet quality (Dörr et al., 2008). However, no published information was available regarding Se supplementation in carnivorous marine finfish fed on SBM-based diets. In the present study, OS supplementation was found to contribute to fish performance, as OS-supplemented diets significantly enhanced the FW, SGR and FCR of fish compared to those fed the same diets without OS supplementation. This finding agrees with the results of previous FM-based studies employing both similar (Le & Fotedar, 2013b ; Le & Fotedar, 2014a) and different species (Abdel-Tawwab et al., 2007 ; Gatlin & Wilson, 1984 ; Han et al., 2011 ; Hilton et al., 1980 ;

Kucukbay et al., 2009 ; Lin & Shiau, 2005 ; Wang et al., 2007 ; Zhou et al., 2009). The present study suggests that Se supplementation is necessary to ensure growth performance of yellowtail kingfish when FM protein in their diets was moderately or substantially replaced with PP ingredients. It is also interesting to note that, though the level of OS supplementation was similar, the Se concentrations in SBM_{25}, SBM_{25+Se}, SBM_{75} and SBM_{75+Se} diets differed, 2.56, 5.53, 1.51, and 4.50 mg kg^{-1} respectively. This was due to the higher Se content of FM than SBM. The effects on fish performance of increased OS supplementation in high SBM diets should be further investigated.

Although fish fed the control diet did not exhibit symptoms of Se deficiency, probably due to the naturally compounded Se available from FM, the biological availability of Se from FM appeared to be low, possibly due to the binding of Se to mercury and other heavy metals (Webster & Lim, 2002). Therefore, Se supplementation may be required to encourage optimal growth, avoid deficiency syndromes and enhance the functional immune system in yellowtail kingfish. Additionally, yellowtail kingfish aquaculture is intended to produce fish fillets for human consumption; thus, it is of considerable interest whether raw feed ingredients can be satisfactorily converted into edible, nutritionally complete fish fillets.

Muscle, kidney and liver tissues are known to store Se in the form of selenomethionine (Se-met) (Thiry et al., 2012) and a linear relationship has been observed between tissue Se and supplemental Se dosage in hybrid striped bass (Cotter et al., 2008). However, Se storage capacity is higher in liver and kidney tissues than in muscle tissue (Elia et al., 2011 ; Hamilton, 2004). In addition, different forms of Se are accumulated in tissues at different levels, with Se-met (organic) retained more than selenite (inorganic) (Le & Fotedar, 2014a ; Lorentzen et al., 1994 ; Wang & Lovell, 1997 ; Zhou et al., 2009). In this study, Se from a commercial product (Sel-Plex®) containing organically bound Se in the form of Se-met was utilised as the feed supplement. The findings showed that fish fed on diets with supplemental Se retained Se in their muscles nearly twice as effectively as those that did not receive the Se supplement. This is consistent with the results of previous studies using similar species (Le & Fotedar, 2013a ; Le et al., 2014b), although slightly higher Se accumulation was observed in the present study, which reflects the

high absorption rate of SBM diets containing Se-met. Moreover, as Se-met is incorporated into proteins in the muscle tissue of marine animals (Maher, 1985), increased accumulation of Se in fish muscles is to be expected.

The major biological form of Se is seleno-cysteine (Se-cys) (Moghadaszadeh & Beggs, 2006). Se-cys is synthesised and specifically incorporated into proteins (Burk & Hill, 1993) through a complex process (McKenzie et al., 2002). Through the Se labelling of mammals *in vivo* or cells in culture, approximately 30–40 seleno-proteins have been detected (Moren et al., 2011), of which 15 have been purified to enable characterisation of their biological functions (Brown & Arthur, 2001). GPx enzymes, a major group of functionally important Se-cys proteins (McKenzie et al., 2002), play an antioxidant role, protecting cells and membranes from the harmful oxidative damage from hydrogen or lipid peroxides (Dhur et al., 1990). Rotruck et al. (1973), moreover, found Se to be an integral structural element of the active core of GPx enzymes in RBCs; these proteins have since been extensively used to measure Se nutritional status.

In the present study, the change of GPx values in response to Se supplementation shows that the Se requirements of yellowtail kingfish were not met by the control diet. Se-dependant GPx activity was significantly higher in the RBCs of fish whose diet was supplemented with Se than in the RBCs of fish fed Se-deficient diets. This result clearly indicates that SBM without Se supplementation provided inadequate Se for maximal GPx activity and thus did not meet yellowtail kingfish Se requirements during the juvenile raising period. The dietary Se requirement of yellowtail kingfish is estimated to be around 5.56 mg kg^{-1} (Le et al., 2014b), which supports the results of the present study. Similarly, in other species, GPx activity is significantly increased with increased dietary Se intake (Lin & Shiau, 2005 ; Liu et al., 2010) and significantly decreased with diets deficient in Se (Bell et al., 1987 ; Gatlin et al., 1986 ; Hilton et al., 1980 ; Wise et al., 1993). Additionally, the GPx levels revealed that Se requirements may increase as a result of handling and confinement stress, such that Se-deficient diets may not meet the increased Se requirements (Rider et al., 2009). The effect of Se on the growth performance of yellowtail kingfish in the present study may be due to the antioxidant role of GPx.

However, feeding a high-SBM diet supplemented with Se to juvenile yellowtail kingfish did not increase GPx activity to the maximum level, although GPx activity was significantly higher in the SBM_{75+Se} group than in the SBM_{75} group. This result may be due to antinutritional phytate interfering with the normal absorption of Se. Commonly applied plant-based feed ingredients such as SBM contain 10–15 g kg^{-1} phytate (Francis et al., 2001). Phytate chelates with di- and trivalent mineral cations, such as Cu, Zn, Co, Mn, Fe, Se, Mg and Ca (Connelly, 2011), consequently forming insoluble complexes in the upper gastrointestinal tract, where maximum mineral absorption typically occurs (Kumar et al., 2012). Several authors have reported reduced levels of minerals such as Ca and Zn in the plasma of common carp due to high phytate (≥1%) diets (Hossain & Jauncey, 1993 ; Papatryphon et al., 1999). Phytate content was not measured in the present study, so the extent to which phytate impacted the absorption of Se and its antioxidant activity in yellowtail kingfish cannot be stated precisely.

The structure and function of cell membranes is strongly correlated with GPx activity (de Almeida et al., 2004 ; Yajima et al., 2009). Presumably, therefore, marine animals with lowered antioxidant activity are more prone to cell damage and decreased oxidation resistance due to reduced Se may profoundly alter the cell membrane structure of fish. For example, previous work with Atlantic salmon (*Salmo salar*) (Poston et al., 1976a) has shown that histological Se deficiency lesions result from inadequate protection against the peroxidation of organelle membrane lipids. In addition, Wang et al. (2013) reported that low dietary Se content aggravates tissue peroxidation and causes acute cell damage in common carp (*Cyprinus carpio*), as shown histopathologically by degeneration and necrosis in the organelles, cells and tissues (including muscle tissue).

Similarly, evidence establishing an association between Se and muscle abnormality was obtained in the present study, as nutritional myopathies and necrosis were observed in the muscle tissue of juvenile yellowtail kingfish fed Se-deficient SBM diets. Most importantly, the findings in the present study suggest that the reduced GPx activity of SBM diets lacking Se supplements, indicative of low cellular Se status, was connected to muscular dystrophy. However, this result must be validated

either by using other plant-derived ingredients or by investigating other carnivorous marine finfish species.

Furthermore, a recent study by Bowyer et al. (2013a) reported that solvent-extracted SBM could replace 10% of FM protein in yellowtail kingfish feed. With similar species, Bowyer *et al*. (2013b) also found that soy protein concentrate (SPC), a highly refined soybean product, was a suitable replacement for FM at a 20% inclusion level. However, it should be noted that no Se supplementation was conducted in those studies. When feed supplementation is discounted from diet evaluation, the appropriate inclusion level of SBM varies widely. For instance, while Silva-Carrillo et al. (2012) reported significant growth deterioration when replacing 20% of FM with SBM in the diet of juvenile spotted rose snapper (*Lutjanus gutatus*), studies have demonstrated that SBM protein can replace from 20% to 30% of FM in the diets of other finfish species, including Mediterranean yellowtail (*Seriola dumerili*) (TomÁS et al., 2005), gilthead sea bream (*Sparus aurata*) (Robaina et al., 1995), cobia (*Rachycentron canadum*) (Zhou et al., 2005) and European sea bass (*Dicentrarchus labrax*) (Dias et al., 1997 ; Lanari & D'Agaro, 2005).

Moreover, in combination with corn gluten meal and meat meal or corn gluten meal and poultry by-product meal, the level of SBM in feed can be increased to 50–60% and 70–90% in juvenile and yearling red sea bream respectively (Aoki et al., 1998 ; Takagi et al., 2000). The discrepancies among findings regarding the incorporation of SBM as an alternative protein source for fish may be due to differences in the quality and treatment of SBM, diet formulation, fish size and culture system, or species-specific responses.

In the present study, it is likely that ANFs restricted the absorption of essential AA substances in SBM-containing diets, resulting in supressed growth when yellowtail kingfish were fed high SBM diets. Reduced ADC of protein was observed in diets containing 75% SBM compared with all other experimental diets. A negative linear relationship between increasing SBM levels and ADC of nutrients has been reported for similar species (Bowyer et al., 2013a); however, the protein ADC values obtained in the present study were higher than those previously reported, which, as mentioned previously, may be related to the presence of ANFs, as mentioned previously. In contrast, several studies have observed similar protein contents for fish fed various

proportions of SBM (Viyakarn et al., 1992 ; Watanabe et al., 1992). Also, with respect to fillet composition, fish fed with diets containing high SBM levels had significantly lower protein content, probably due to the poor growth performance associated with these diets.

4.5. Summary

In summary, the present study demonstrates that Se supplementation promotes improved growth, feed utilisation, antioxidant activity and histological performance in juvenile yellowtail kingfish when SBM proteins are included in the diets. These findings may assist the formulation of cost-effective, ecologically-friendly practical diets for yellowtail kingfish. Notably, this study is the first to address the importance of adding Se to SBM-based diets in carnivorous marine finfish species. Further work is required to determine whether increased Se supplementation and/or inclusion of more refined plant-derived proteins can improve the substitution level of PP ingredients in fish diets.

CHAPTER 5: Effects of organic selenium supplementation on growth, accumulation, haematology and histopathology of juvenile barramundi (*Lates calcarifer*) fed high soybean meal diets

This chapter has been published in Biological Trace Element Research
Volume 174 (2016): 436-447

5.1. Introduction

The use of fishmeal (FM) as the major protein source for aquaculture production has been plagued by both economic and environmental objections. While FM is disputably sustainable (Sarker et al., 2013 ; Troell et al., 2013), increased demand for FM from rapidly expanding aquaculture can be expected to remain high in the long term (Globefish, 2015 ; Villasante et al., 2013). Traditionally, carnivorous fishes perform well on diets high in FM, which contain high protein levels and provide a remarkably good source of essential amino acids (AA), essential fatty acids, phospholipids, nucleotides, macro- and micro-elements (Tacon & Metian, 2009). Nevertheless, the substitution of FM with lower-cost, widely available plant protein (PP) ingredients such as soybean meal (SBM) would be likely to improve the sustainability of the aquaculture industry greatly. Moreover, aquafeeds represent the largest portion of the production cost, with FM being the most expensive ingredient. Hence, efforts to find economically viable and environmentally friendly feeds never cease, with researchers and industries continually working to expand their sourcing.

SBM is promising as a PP replacement for FM, and its utilisation in aquaculture feeds is generally recognised. Despite SBM being the most widely utilised plant protein (PP) source in aquaculture feed today, FM remains the primary protein source for most carnivorous aquaculture species (Tacon & Metian, 2015) . One of the problems with SBM use is the existence of antinutritional factors (ANF), such as trypsin inhibitors, saponin, and phytic acid (Krogdahl et al., 2010 ; Kumar et al., 2012).

Phytic acid, or phytate in salt form, is the major storage form of phosphorus (P) in plant protein (PP) seeds, accounting for 50–80% of the total P (Harland & Morris, 1995). Phytate-bound P cannot be digested by monogastric animals, including fish.

In its native state, phytic acid is a strong chelator of divalent minerals, such as copper (Cu), zinc (Zn), iron (Fe), magnesium (Mg), calcium (Ca), and selenium (Se) (Connelly, 2011 ; Sathe & Reddy, 2002). Phytate, when present in high amounts in the diet, has been demonstrated to inhibit growth, feed utilisation, and protein and mineral bioavailability (Storebakken et al., 1998 ; Usmani & Jafri, 2002). However, organic chelated minerals have been reported to be able to resist these effects with the use of mineral inhibitors, which increase mineral bioavailability to fish (Ashmead, 1992).

Supplementing a fish's diet with these organic sources provides a degree of support to the intestinal absorption of trace minerals as they counteract the interference of substances that establish insoluble complexes with ionic trace elements (Apines-Amar et al., 2004). Cotter (2006) reported that hybrid striped bass (*Morone saxatilis* × *M. chrysops*) juveniles fed on SBM or casein-based diets without Se supplementation showed lowered growth and antioxidant glutathione peroxidase (GPx) activity compared with those on the FM-based diets. Fontagne-Dicharry et al. (2015) evaluated the effects of supplementing diets based on mixtures of PPs with organic and inorganic sources in rainbow trout (*Oncorhynchus mykiss*), and organic Se showed a greater availability than inorganic sources. They also concluded that the addition of Se to a high proportion of PP in the diet enhanced the antioxidant status of fish. Moreover, in a FM-based diet study, Le and Fotedar (2014a) found that juvenile yellowtail kingfish (*Seriola lalandi*) fed diets containing Se-organic chelate retained a higher level of muscle Se than fish fed diets containing selenite.

Although only required in trace amounts, Se is an essential nutrient for normal growth, physiological function, and cellular metabolism in fish (Gatlin et al., 1986 ; Watanabe et al., 1997). Se serves as an important component for the regulation of the antioxidant enzyme GPx, which protects membranes at both the cellular and subcellular level from oxidative damage (Jaramillo Jr et al., 2009). Fish may absorb minerals directly from the surrounding water, but diet is the primary source of Se (Lall, 2003). Recently, supplementing diets with Se has been shown to have beneficial effects on the growth, physiological and health responses of a variety of fish species (Lin & Shiau, 2005 ; Jaramillo Jr et al., 2009 ; Kucukbay et al., 2009 ; Arshad et al., 2011 ; Han et al., 2011 ; Wang et al., 2013 ; Le & Fotedar, 2014b).

Organic trace elements have been employed in the aquaculture of several fast growing species, yet, their effect on slower growing species remains unknown.

Barramundi (*Lates calcarifer*), also known as Asian seabass, is a carnivorous fish that is broadly spread throughout the Asia–Pacific region. The species has been an economically important species in regions such as southeast Asia and Australia (Paul et al., 2013). Like other carnivorous species, outstanding growth performance is achieved when the fish are fed on an FM diet. Recently, a considerable amount of work has been dedicated to investigating the potential incorporation of PP ingredients in the diets of barramundi (Glencross, 2006 ; Glencross et al., 2011 ; Katersky & Carter, 2009 ; Ngo et al., 2015 ; Tabrett et al., 2012 ; Van Vo et al., 2015). However, less attention has been paid to the species' trace mineral nutrition when plant feedstuffs are used as the major source of protein. The present study aims to fill that gap by evaluating the effect on growth, accumulation, antioxidant status and histopathology in juvenile barramundi when SBM-based diets are supplemented with organic Se (OS).

5.2. Materials and methods

Two separate feeding trials (Trial 1 and Trial 2) were carried out at Curtin Aquatic Research Laboratory (CARL), Curtin University, Perth, Australia. Trial 1 evaluated the effects of OS supplementation in SBM diets for barramundi, whereas Trial 2 investigated the potential effect of dietary supplementation of OS when a high proportion of SBM was replacing FM in the barramundi diet.

5.2.1. Trial 1

Diet and experimental design

All ingredients used in the study were sourced from a commercial supplier (Specialty Feeds, Glen Forrest, Western Australia). SBM was ground to powder in a food processor. All ingredients were thoroughly blended into a homogenous mixture. Fish oil and 30% distilled water were poured into the premixed dry ingredients and mixed for 15 min in a food mixer. The mixture was then pelleted into 2-mm dye with a laboratory pelleting machine. The pellets were air-dried and refrigerated at −20ºC until fed. Formulation and proximate analyses of the experimental diets and amino acid (AA) profile of the major ingredients are shown in Table 5.1 and Table 5.2.

Three isoproteic (48% crude protein) and isocaloric (20 MJ kg^{-1} gross energy) basal diets were formulated to contain 0%, 15% and 43% SBM as replacement for 0%, 25% and 75% FM protein: each either remained unsupplemented or was supplemented with 2 g OS kg^{-1} (SBM_0, SBM_{0+OS}, SBM_{25}, SBM_{25+OS}, SBM_{75}, SBM_{75+OS}). The supplementation levels of OS were selected based on the supplementation level for marine finfish as proposed by Le and Fotedar (2013b), which was below an estimated threshold level of ~4 mg Se kg^{-1} as recommended by NRC (2011). The diets without OS supplementation were regarded as the Se-deficient group.

Fish and experimental conditions

A total of 360 healthy juvenile barramundi (mean weight of 5.20 g) were obtained from the Australian Centre for Applied Aquaculture Research, Fremantle, Western Australia (WA), Australia. Fish were acclimated to feeding and rearing conditions for seven days before the commencement of the trial. During the acclimation period, all fish were fed the commercial diet twice daily. Each tank was supplied with recirculated water from an external biofilter (Fluval 406, Hagen, Italy) at a rate of 10 L min^{-1}. Water flow rates were monitored daily to maintain similar water exchange in the tanks. All experimental tanks were equipped with constant aeration and pure oxygen (compressed oxygen, BOC, Perth, WA). Water quality parameters such as temperature, dissolved oxygen, and salinity were measured daily and maintained at 27-29 ºC, >5 mg L^{-1}, 32-34 ppt, respectively. The photoperiod was set at 12 hours of fluorescent light per day, as suggested by Bermudes et al. (2010).

Prior to the feeding experiment, fish were starved for 24 h, bulk-weighed and then randomly assigned to 18, 300-L experimental tanks at the density of 20 fish $tank^{-1}$, with three replicates per dietary treatment. During the feeding trial, fish were fed the respective experimental diets *ad libitum* during two feeding sessions a day at 0900 h and 1500 h for 60 days. Fish were bulk-weighed every 15 days to record growth. Dead fish were weighed and recorded in order to adjust the calculation of the feed conversion ratio (FCR) and survival.

Table 5.1. Formulation and composition of the experimental diets

Ingredients[a] (g kg^{-1} DM)	Diets					
	SBM_0	SBM_{0+OS}	SBM_{25}	SBM_{25+OS}	SBM_{75}	SBM_{75+OS}
Fishmeal	460	460	340	340	180	180
Soybean meal[b]	-	-	150	150	430	430
Wheat gluten	100	100	100	100	100	100
Wheat flour	80	80	30	30	10	10
Casein	120	120	120	120	120	120
Fish oil	100	100	110	110	110	110
Wheat starch	65	63	50	50	25	23
Cellulose	50	48	75	73	-	-
Se-free premix[c]	20	20	20	20	20	20
Organic selenium[d]	-	2	-	2	-	2
Chromic oxide	5	5	5	5	5	5
Proximate content (*%*)						
Dry matter	85.17	85.20	88.65	89.21	90.38	89.72
Ash	8.25	8.22	7.86	7.73	7.43	7.22
Protein	49.28	49.21	49.08	49.15	49.30	49.16
Lipid	15.06	15.03	14.95	14.94	14.52	14.55
Gross energy (MJ/kg)	21.33	21.30	22.16	22.10	20.13	20.07
Se (mg kg^{-1})	3.15	5.12	2.56	4.52	1.53	3.51

[a] Supplied by Specialty Feeds, Perth, WA, Australia, except for Sel-Plex and chromic oxide, obtained from AllTech, Lexington, Kentucky, USA and Thermo Fisher Scientific, Scoresby, Vic, Australia, respectively.

[b] Solvent-extracted; Malaysian origin

[c] Contains the following (as g kg^{-1} of premix): iron, 10; copper, 1.5; iodine, 0.15; manganese, 9.5; zinc, 25; vitamin A retinol, 100 IU; vitamin D3, 100 IU; vitamin E, 6.25; vitamin K, 1.6; vitamin B1, 1; vitamin B2, 2.5; niacin, 20; vitamin B6, 1.5; calcium, 5.5; biotin, 0.1; folic acid, 0.4; inositol, 60; vitamin B12, 0.002; choline, 150; ethoxyquin, 0.125.

[d] Sel-Plex® (AllTech, Lexington, Kentucky, USA)

Table 5.2. Hydrolysed amino acid composition of FM, SBM, wheat gluten and casein (g 100 g^{-1} protein)

Amino acid	FM	SBM	Wheat gluten	Casein
Essential				
Arginine	4.35	3.69	1.50	3.32
Histidine	2.09	1.23	0.96	2.73
Isoleucine	3.04	2.25	2.35	4.70
Leucine	5.17	3.88	9.75	8.70
Lysine	4.77	2.83	0.80	7.46
Methionine	1.86	0.58	1.05	2.19
Phenylalanine	2.87	2.61	3.47	4.67
Threonine	3.19	1.89	1.76	3.71
Valine	3.26	2.14	2.58	5.75
Non-essential				
Alanine	4.42	2.04	NA	2.56
Aspartic acid	6.20	5.48	NA	6.24
Glutamic acid	8.25	8.96	NA	18.60
Glycine	4.73	1.94	NA	2.38
Proline	3.81	3.01	NA	8.80
Serine	3.05	2.64	NA	5.36

NA: not analysed

5.2.2. Trial 2

Diet and experimental design

To investigate the potential improvement of elevated OS levels in high SBM diets, five experimental diets were prepared. The SBM_{75+OS} diet was used as the basal diet (SSe_1). Other diets were supplemented with 3 (SSe_2), 4 (SSe_3), 5 (SSe_4), and 7(SSe_5) mg Se kg^{-1}. The measured Se concentrations of the experimental diets were 3.51 mg Se kg^{-1} (SSe_1), 4.47 mg Se kg^{-1} (SSe_2), 5.48 mg Se kg^{-1} (SSe_3), 6.48 mg Se kg^{-1} (SSe_4), and 8.50 mg Se kg^{-1} (SSe_5). Diets were prepared in the same way as in Trial 1.

Fish and experimental conditions

A total of 300 healthy juvenile barramundi (mean weight of 8.2 g) were obtained from the Australian Centre for Applied Aquaculture Research, Fremantle, Western Australia (WA), Australia. The experimental conditions followed the conditions described for Trial 1.

5.2.3. Sampling and analytical methods

All fish were starved for 24 hours prior to final sampling to achieve a basic metabolite state. The fish were then anaesthetised with tricaine methanesulfonate (MS-222, Sigma-Aldrich, Castle Hill, NSW, Australia) at 100 mg L^{-1} and weighed individually. Blood samples from six fish in each tank were then taken by caudal vein puncture with a 1 mL disposable syringe. The extracted blood was transferred to a heparinised tube for haematological analysis. Haematocrit and leucocrit were determined by centrifugation of capillary glass tubes, using McLeay and Gordon's method (1977). An Hb kit (Randox Laboratories, Antrim, United Kingdom) was used to measure the haemoglobin (Hb) content. Erythrocyte GPx activity was quantitatively assayed using the Ransel RS-505 kit (Randox, Antrim, United Kingdom) and a chemistry immune analyser (AU400, Olympus, Tokyo, Japan) at 340 nm and 37 °C. The results were expressed as units of GPx g Hb^{-1}.

The proximal compositions of the diet were determined based on Association of Official Analytical Chemists procedures (AOAC, 1990). Dry matter was determined by oven drying to constant weight at 105ºC; crude ash by combustion at 550 ºC; crude protein content (N × 6.25) by the Kjeldahl digestion method; crude lipid content by the Soxhlet technique; and gross energy content by an IKA oxygen bomb calorimeter (Heitersheim, Germany). The AA content of the diets was determined after samples were hydrolysed in HCl (Barkholt & Jensen, 1989 ; Rayner, 1985). Analyses were performed on an Agilent 1100 series high-performance liquid chromatography system (HPLC, Agilent Technologies, Germany) using conditions similar to those described by Gratzfeld-Huesgen (1998).

The total Se in diet and muscle tissue samples was determined using an aqua regia digestion with reduction precipitation (Dulski, 1996). Essentially, the sample was dissolved in aqua regia, filtered, and the selenium reduced to the zero oxidation state

where it precipitated. The precipitate was collected using filtration, dissolved in acid and read on an inductively coupled plasma-mass spectrometry instrument(ICP-MS). A mass of 5 g was catch-weighed in a beaker. Nitric acid (HNO_3) and hydrochloric acid (HCl) were added sequentially to make aqua regia. The excess acid was boiled off and the digested sample was leached in HCl. The sample was filtered, the residue and beaker were thoroughly washed, and the filtrate was collected. The selenium was precipitated using a Cu solution in a mixture of citric and ascorbic acids at a low temperature. The selenium was filtered off and dissolved in nitric acid. HCl and deionised water were added and the solution was mixed and read on a calibrated ICP-MS.

5.2.4. Histopathology

All histopathological assessments were accomplished using standard laboratory procedures. At the end of the trial, segments of muscle, liver and kidney from six fish in each tank were fixed in 10% buffered formalin, dehydrated in ethanol before equilibration in xylene and embedding in paraffin wax. Sections of approximately 5 µm were cut and stained with haematoxylin and eosin for histological observation under a light microscope (BX40F4, Olympus, Tokyo, Japan). Light microscopy samples were prepared according to standard histological techniques (Luna, 1968).

5.2.5. Calculations

Growth and feeding performances were measured using the calculated parameters below:

$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100 \times \left[\frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{days}}\right]$$

$$\text{Weight gain (\%)} = 100 \times \left[\frac{\text{final weight} - \text{initial weight}}{\text{initial weight}}\right]$$

$$\text{Feed intake (FI, g fish}^{-1}\text{ days}^{-1}) = \left[\frac{\text{dry diet given} - \text{dry remaining diet recovered}}{\text{number of fish}}\right] / \text{days}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake}}{\text{weight gain}}$$

$$\text{Survival (S, \%)} = 100 \times \left[\frac{\text{final number of fish}}{\text{initial number of fish}}\right]$$

$$\text{Thermal growth coefficient (TGC)} = \left[\frac{(\text{final weight}^{1/3} - \text{initial weight}^{1/3})}{\text{temperature℃} \times \text{days}}\right] \times 1000$$

5.2.6. Statistical analysis

In Trial 1, treatment effects were analysed by factorial analysis of variance (ANOVA), in which the factors were SBM protein (control FM, low or high) and OS (unsupplemented or supplemented) using the General Linear Model procedure available on SPSS software (version 22, IBM, Australia). If a significant interaction was observed between these two factors ($P < 0.05$), data were then analysed for differences between mean values using Duncan's multiple range test and means were considered to be significantly different when $P < 0.05$. Percentage data were computed using arcsine transformations. In Trial 2, the data were analysed using one-way ANOVA (SPSS version 22, IBM, Australia). Significant differences between means were evaluated using the Duncan's post hoc test. Probabilities of $P < 0.05$ were considered significant. Linear regression analysis (Microsoft Excel 2010 for Windows) was employed to plot muscle Se accumulation versus dietary Se concentration.

5.3. Results

5.3.1. Trial 1

Growth and survival

Mortalities recorded during the trial were lower than 4% and those due to cannibalism occurred within the first week. All fish exhibited similar feeding behaviour, promptly accepting the feed during the course of the trial. The overall growth performance and feed utilisation by the fish are shown in Table 5.3. There was no interaction effect for any of the measurements. However, either inclusion of SBM or supplementation of OS had a significant effect ($P < 0.05$) on FW, SGR and WG. FW, SGR and WG decreased as the level of SBM increased. Regardless of SBM inclusion, FW, SGR, WG, FCR and TGC were significantly improved by OS supplementation ($P < 0.05$).

Haematology and antioxidant enzyme

The haematology and GPx activity of barramundi fed different SBM and OS levels are presented in Table 5.4. No significant differences were observed in Hb, haematocrit or leucocrit content for any of the dietary treatments. GPx activities were significantly higher in fish fed OS-supplemented diets ($P < 0.05$).

Table 5.3. Performance of juvenile barramundi fed different SBM levels with and without OS supplementation for 60 days[a]

Parameters	0 g kg^{-1} OS			2 g kg^{-1} OS			Two-way ANOVA (*P* value)		
	SBM_0	SBM_{25}	SBM_{75}	SBM_0	SBM_{25}	SBM_{75}	SBM	OS	SBM × OS
FW	28.08 ± 0.73	25.92 ± 0.66	24.95 ± 0.71	28.62 ± 1.01	28.45 ± 0.80	26.74 ± 0.56	0.005	0.007	0.314
SGR	2.81 ± 0.06	2.64 ± 0.14	2.60 ± 0.09	2.87 ± 0.23	2.83 ± 0.25	2.74 ± 0.08	0.025	0.009	0.488
WG	439.9 ± 3.75	386.9 ± 3.22	378.0 ± 2.89	459.6 ± 2.68	446.7 ± 1.45	418.9 ± 2.36	0.022	0.009	0.475
FI	0.46 ± 0.03	0.44 ± 0.01	0.42 ± 0.03	0.47 ± 0.02	0.46 ± 0.01	0.46 ± 0.02	0.056	0.088	0.292
FCR	1.22 ± 0.23	1.28 ± 0.15	1.29 ± 0.13	1.19 ± 0.15	1.16 ± 0.18	1.24 ± 0.22	0.052	0.005	0.218
S	98.3 ± 1.67	100 ± 0.00	98.3 ± 1.67	100 ± 0.00	98.3 ± 1.67	96.7 ± 3.31	0.482	0.593	0.685
TGC	0.756 ± 0.05	0.694 ± 0.02	0.690 ± 0.17	0.791 ± 0.21	0.765 ± 0.19	0.754 ± 0.07	0.084	0.010	0.725

[a] Means of three replicates ± SE.

SBM_0, SBM_{25}, SBM_{75} (fishmeal protein replaced by 0%, 25% and 75% SBM protein respectively).

FW (final weight, g); SGR (specific growth rate, % body weight day^{-1}); FI (feed intake, g $fish^{-1}$ day^{-1}); WG (weight gain, %);

FCR (feed conversion ratio); S (survival, %); TGC (thermal growth coefficient, day 1–60; 28.7ºC).

Table 5.4. Haematology and GPx activity of barramundi fed different SBM levels with and without OS supplementation for 60 days[a]

Parameters	0 g kg^{-1} OS			2 g kg^{-1} OS			Two-way ANOVA (*P* value)		
	SBM_0	SBM_{25}	SBM_{75}	SBM_0	SBM_{25}	SBM_{75}	SBM	OS	SBM × OS
Haemoglobin	73 ± 3.31	74 ± 2.88	75 ± 2.78	74 ± 3.16	73 ± 2.92	76 ± 2.77	0.597	0.076	0.597
Haematocrit	31.60 ± 0.08	31.77 ± 0.16	30.61 ± 0.24	33.87 ± 0.78	34.06 ± 0.55	33.42 ± 0.42	0.369	0.001	0.903
Leucocrit	1.36 ± 0.28	1.35 ± 0.18	1.28 ± 0.09	1.33 ± 0.33	1.34 ± 0.27	1.32 ± 0.12	0.774	0.414	0.765
GPx	180 ± 2.65	178 ± 1.71	173 ± 1.11	193 ± 2.80	190 ± 1.73	182 ± 1.69	0.002	0.000	0.705

[a] Means of three replicates ± SE.

SBM_0, SBM_{25}, SBM_{75} (fishmeal protein replaced by 0%, 25% and 75% SBM protein respectively).

Hb (g dL^{-1}); Haematocrit (%); Leucocrit (%), GPx (U/g Hb); Muscle Se (μg/g).

Tissue Se accumulation

After the 60-day feeding trial, fish fed with diets supplemented with OS attained significantly higher Se content in their muscle tissues. A linear relationship between dietary Se concentration and tissue Se content was discovered, as shown in Figure 5.1.

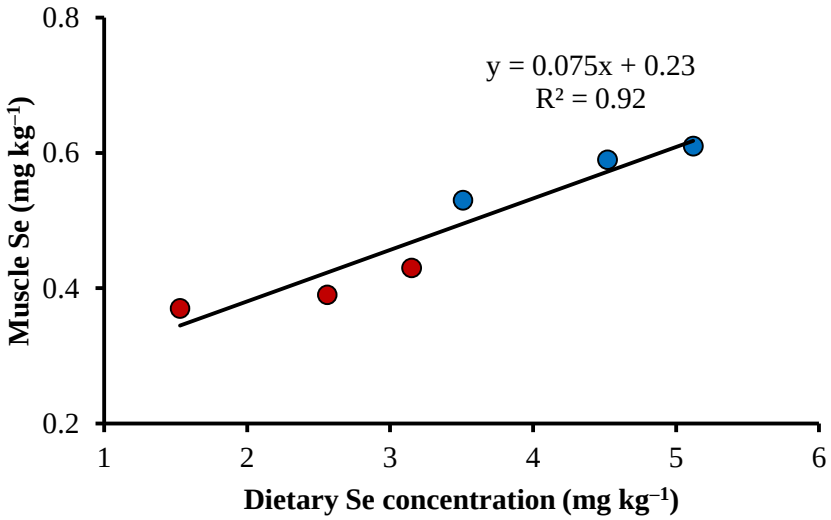

Figure 5.1. Linear relationship between dietary Se concentration and tissue Se. Note blue markers that represent OS-supplemented dietary treatments and red markers for dietary treatments without OS supplementation.

Histopathology assay

Both liver and kidney tissue was histologically normal. However, histological abnormalities were found in the muscle of fish fed the diets lacking OS supplementation. Figure 5.2 demonstrates the severity of the muscle injury seen in OS-deficient group. Normal muscle structure in cross-section is characterised by rounded, densely packed, and uniformly identical muscle fibres (Figure 5.2a). Severe histological alterations included falsification, disconnection, and longitudinal rupture of muscle fibres, which are typical signs of Se deficiency-induced myopathy (Figure 5.2b).

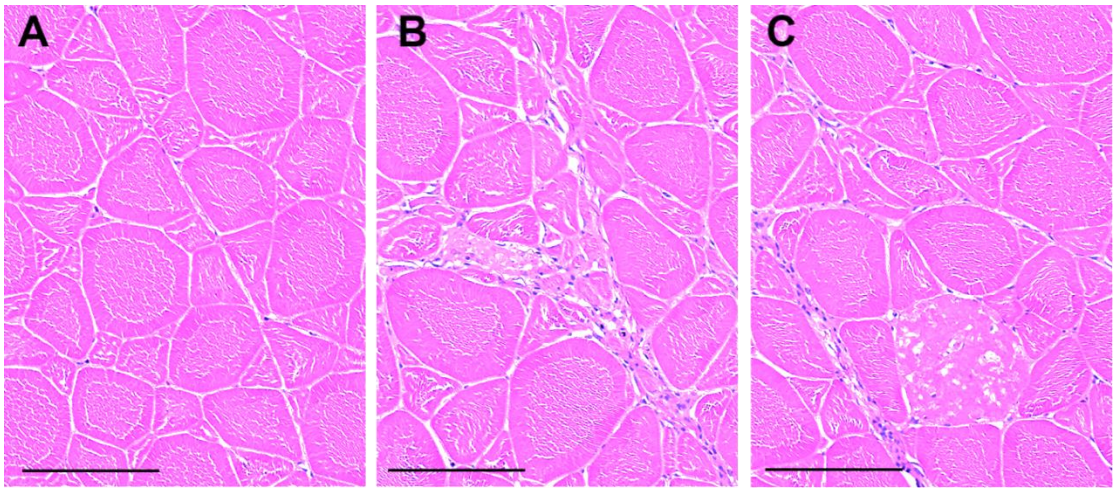

Figure 5.2. Cross-section of the muscle of barramundi diets displaying normal histological structures (A) observed in Se-supplemented dietary group, and Se deficiency-induced myopathy (B, C) found in Se-deficient dietary group. Note severe muscle degeneration and hypercontraction of the surrounding muscular fibres (arrows) (scale bar = 100 μm).

5.3.2. Trial 2

Growth and survival

The highest FW, SGR and WG were achieved by fish fed the SSe_3 diet, along with those fed the SSe_1 and SSe_2 diets (Table 5.5). Fish fed SSe_4 and SSe_5 diets had significantly lower FW, SGR and WG compared with fish fed the other diets. FI was significantly affected by the level of OS in the diet ($P < 0.05$). Lower FI was observed for the fish fed the SSe_5 diet, although it was not significantly different from that of the SSe_1 diet. FCR was not improved by the addition of OS in high SBM diets. Instead, high FCR was observed in fish fed SSe_4 and SSe_5 diets. However, dietary OS level significantly affected the TGC of fish ($P < 0.05$). Fish fed the diets supplemented with 7 mg Se kg^{-1} (SSe_5) had a TGC of 0.652 compared to 0.698-0.755 for fish fed other diets. Survival was high, exceeding 96%, during the feeding trial.

Table 5.5. Performance of juvenile barramundi fed different SBM levels supplemented with various OS levels for 60 days[a]

Se supplementation levels (mg Se kg^{-1})	FW	SGR	WG	FI	FCR	S	TGC
SSe_1 (3.51)	34.87 ± 2.33^{bc}	2.43 ± 0.33^{b}	329.8 ± 4.25^{c}	0.54 ± 0.02^{ab}	1.22 ± 0.03^{a}	98.3 ± 1.67	0.728 ± 0.11^{bc}
SSe_2 (4.47)	35.70 ± 1.08^{c}	2.45 ± 0.19^{b}	334.3 ± 5.01^{c}	0.60 ± 0.15^{b}	1.30 ± 0.02^{ab}	96.7 ± 3.31	0.755 ± 0.25^{c}
SSe_3 (5.48)	35.91 ± 2.01^{c}	2.48 ± 0.15^{b}	343.5 ± 1.18^{c}	0.58 ± 0.09^{b}	1.26 ± 0.01^{a}	96.7 ± 3.31	0.739 ± 0.09^{bc}
SSe_4 (6.48)	33.42 ± 3.17^{ab}	2.34 ± 0.24^{a}	308.3 ± 3.37^{b}	0.57 ± 0.12^{b}	1.33 ± 0.06^{bc}	98.3 ± 1.67	0.698 ± 0.37^{b}
SSe_5 (8.50)	30.97 ± 1.80^{a}	2.20 ± 0.29^{a}	273.2 ± 4.38^{a}	0.53 ± 0.22^{a}	1.37 ± 0.07^{c}	96.7 ± 1.67	0.652 ± 0.85^{a}

[a] Means of three replicates ± SE.

FW (final weight); SGR (specific growth rate); WG (weight gain, %); FI (feed intake); FCR (feed conversion ratio); S (survival, %); TGC (thermal growth coefficient, day 1–60; 28.3ºC).

Values in the same column without a common superscript are significantly different ($P < 0.05$).

Haematology and antioxidant enzyme

Haematology and GPx activity data are shown in Table 5.6. The effect of OS supplementation on haematological indices was observed. Although no significant differences were shown in Hb among the dietary treatments, significantly higher haematocrit was found in fish receiving 2, 3 and 4 mg OS kg^{-1} ($P < 0.05$). Haematocrit decreased significantly as the OS supplementation level increased to 5 and 7 mg Se kg^{-1} ($P < 0.05$). Similarly, lower levels of leucocrit were observed in fish fed the SSe_4 and SSe_5 diets ($P < 0.05$). The highest GPx value was observed in juvenile barramundi fed the SSe_3 diet, but this value was not significantly different from that of fish fed the SSe_2 and SSe_4 diets.

Tissue Se accumulation and histopathology assay

The Se content of the muscle tissue significantly increased with as the OS supplementation level was increased in the diets ($P < 0.05$); however, no significant differences were found in the SSe_4 and SSe_5 diets ($P > 0.05$). The Se content of the muscle tissue for the differently fed fish is shown in Table 5.6. Histopathological alterations were not observed in liver or muscle tissues in any of the fish examined after 60 days.

Table 5.6. Haematology, GPx activity and muscle Se of barramundi fed different SBM levels with various OS levels for 60 days[a]

Se supplementation levels (mg Se kg^{-1})	Hb	Haematocrit	Leucocrit	GPx	Muscle Se
SSe_1 (3.51)	76 ± 2.45	33.87 ± 1.41[b]	1.40 ± 0.38[b]	181.7 ± 1.39[a]	0.56 ± 0.02[a]
SSe_2 (4.47)	79 ± 1.80	34.27 ± 1.57[b]	1.48 ± 0.11[b]	185.3 ± 2.17[abc]	0.62 ± 0.02[b]
SSe_3 (5.48)	79 ± 2.30	36.06 ± 0.83[b]	1.33 ± 0.34[ab]	187.6 ± 1.17[c]	0.67 ± 0.01[c]
SSe_4 (6.48)	78 ± 1.77	30.77 ± 1.62[a]	1.23 ± 0.48[a]	185.7 ± 1.56[bc]	0.71 ± 0.01[d]
SSe_5 (8.50)	76 ± 1.98	30.73 ± 1.57[a]	1.20 ± 0.33[a]	184.5 ± 3.44[ab]	0.73 ± 0.01[d]

[a] Means of three replicates ± SE.

Hb (%); Haematocrit (%); Leucocrit (%), GPx (U/g Hb); Muscle Se (μg/g).

Values in the same column without a common superscript are significantly different ($P < 0.05$).

5.4. Discussion

In the present study, growth performance of juvenile barramundi was independently affected by the SBM protein and the OS supplementation but not by their interaction. Irrespective of OS level, the FW, SGR and WG of the fish significantly decreased as dietary SBM increased, which is in accordance with the results reported for cobia (*Rachycentron canadum*) (Chou et al., 2004 ; Zhou et al., 2005), cuneate drum (*Nibea miichthioides*) (Wang et al., 2006b) and gilthead sea bream (*Sparus aurata*) (Nengas et al., 1996). The studies of Bowyer et al. (Bowyer et al., 2013a) reported that SBM inclusion level above 20% caused growth retardation in juvenile yellowtail kingfish (*S. lalandi*). In addition, when SBM was increased above 50% in rainbow trout (*O. mykiss*) diets, growth was adversely affected (Barrows et al., 2007). On the other hand, 44% FM protein could be substituted with SBM in diets for Egyptian sole (*Solea aegyptiaca*) without hampering their growth (Bonaldo et al., 2006). Further, fish performance was not negatively influenced when FM protein was replaced entirely with SBM in the diets for red sea bream (*Pagrus major*) (Kader et al., 2012). Inconsistencies in results concerning the utilisation of SBM as a protein source for fish may be attributed to the diet quality, diet composition, variations in fish size, and culture conditions.

All fish readily accepted the experimental diets during the 60-day feeding trial. Although PP has often been associated with palatability issues (Arndt et al., 1999 ; Kissil et al., 2000), no difference in FI was observed between dietary treatments in the present study. Indeed, although growth indices were significantly lower in those consuming higher SBM diets, FCR remained unaffected. Reduction in growth performance of the fish fed diets containing SBM may be due to the chelation of antinutritional phytate with mineral ions and protein complexes, which reduces the bioavailability of minerals and proteins (Kumar et al., 2012). Phytate also caused anomalies on the ephitelial layer of the pyloric caeca of the intestine (Richardson et al., 1985). However, despite the low level of FM, the SGR and feed utilisation are comparable to those previously reported for barramundi of similar size (Van Vo et al., 2015). These results thus agree with earlier findings that barramundi appear to have a high tolerance for PP sources (Ngo et al., 2015 ; Tabrett et al., 2012 ; Van

Vo et al., 2015), with the proposed threshold value of FM being 15% of the feed (Glencross et al., 2011).

The present data show that, regardless of SBM levels, the supplementation of dietary OS enhances the growth of barramundi. Differences in the growth seem to be associated with variations in feed efficiency, since the FI was identical in both Se-deficient and Se-supplemented groups. The beneficial effect of OS on feed efficiency has also been reported for land animals such as broiler chickens (*Gallus gallus*) (Wang & Xu, 2008). Zhou et al. thus hypothesise that a downturn of protein synthesis is responsible for delay in growth performance in Se-deficient fish (Zhou et al., 2009). Furthermore, impaired growth in barramundi receiving OS-deficient SBM-based diets was substantiated by typical Se-deficiency syndromes, such as skeletal myopathy. The better FW, SGR, WG and TGC of fish fed diets supplemented with OS (3.51-5.12 mg Se kg^{-1}) compared to those fed diets devoid of OS supplementation (1.52-3.15 mg Se kg^{-1}) indicates that Se derived from FM or PP alone cannot fulfil the requirements of the fish.

In line with the results of the present study, the dietary Se requirement of yellowtail kingfish (*S. lalandi*) was not satisfied by Se derived from a FM-based diet (Le & Fotedar, 2013b), and organic selenomethionine (Se-Met) supplementation enhanced the growth and antioxidant capacity of the fish (Le & Fotedar, 2014a). Enhancement of WG with dietary OS supplements was also demonstrated in grouper (*Epinephelus malabaricus*) (Lin & Shiau, 2005) and marron (*Cherax cainii*) (Nugroho & Fotedar, 2013). Furthermore, Penglase et al. (2010) investigated whether rotifers fed Se-enriched yeast (3 mg Se kg^{-1}) can enhance larval performance in Atlantic cod (*Gadus morhua*), concluding that rotifers require additional Se supplementation to fulfil the larval cod requirements. Furthermore, it is commonly acknowledged that OS sources such as Se-Met and seleno-yeast have higher bioavailability than inorganic Se sources for fish (Jaramillo Jr et al., 2009 ; Wang & Lovell, 1997). Correspondingly, dietary Se is required at a subordinate level when sourcing OS. Hence, the requirement for dietary Se of juvenile barramundi might be further increased by a certain extent when inorganic Se is added. More work is needed to examine this.

Haematological indices are a beneficial tool that can be used to assess the health status and physiological condition of fish (Adams et al., 1993 ; Anderson et al.,

1996). In the present study, there was no obvious linkage between the substitution level of FM with SBM in the diet and the relapse of haematological indicators of barramundi. What is more, supplementation of OS in the diets improved the haematocrit concentration of the fish. The average haematocrit concentration for barramundi in the current study ranged from 30.61 to 31.77 % (OS-deficient group) and from 33.42 to 34.06 % (OS-supplemented group), all of which were within the normal range (30–45%), as suggested by Adams et al. (1993).

The sensitivity of haematological parameters to variation in nutritional properties, including Se, has been reported in several species. A significant increase in the haematological conditions was induced by Se supplements in the diets of yellowtail kingfish (*S. lalandi*) (Le et al., 2014a) and African catfish (*Clarias gariepinus*) (Abdel-Tawwab et al., 2007). Dietary Se at 0.62 mg Se kg^{-1} decreased the red blood cell (RBC) count and the Hb of loach (*Paramisgurnus dabryanus*) (Hao et al., 2014). Furthermore, it was proposed that the reduction in the RBC, haematocrit and Hb corresponded to the growth depression of red sea bream (*P. major*) following waterborne Se exposure higher than 100 μg L^{-1} (Kim & Kang, 2014). It seems that a number of abnormalities associated with the stress and disease of cultured fish are directly or indirectly related to pathophysiological changes in the blood system (Wedemeyer, 1996).

Se exerts its biological roles via selenoproteins (Hoffmann & Berry, 2008), and its most crucial function is its antioxidant capacity, enabling the formation of selenocysteine (Se-Cys), part of the active core of GPx (Köhrle et al., 2000). GPx is an essential antioxidant that affords the first line of protection of the enzymatic defence system against oxidative stress (Ashouri et al., 2015), a pathophysiological condition that injures membrane and cellular structures through the constant release of reactive oxygen species (ROS), which can be induced by both biotic and abiotic stressors (Neve & Richard, 1994). Se functions as a catalyst for the removal of hydrogen peroxides and lipid hydroperoxides in the cell cytosol and mitochondrial matrix (Brown & Arthur, 2001). These actions promote membrane integrity and provide protection to biomolecules, such as lipids, lipoproteins and DNA, against the proliferation of further oxidative damage (Yamashita et al., 2010). GPx activity ties in with either dietary Se level or tissue Se concentration in fish except at the

extremes (Elia et al., 2011 ; Gatlin & Wilson, 1984). In the present study, the GPx activity was significantly higher in the OS-supplemented group than in the OS-deficient group. This result coincided with that of growth performance, suggesting that OS-deficient fish might have been incapable of coping with the prooxidant stress caused by the reduction in the GPx antioxidant activity, thus decelerating the growth.

A reduction in GPx activity induced by Se-deficient diets has been reported for a range of species such as Atlantic salmon (*S. salar*) (Bell et al., 1987), gibel carp (*Carassius auratus gibelio*) (Han et al., 2011), grouper (*E. malabaricus*) (Lin & Shiau, 2005), and yellowtail kingfish (*S. lalandi*) (Le & Fotedar, 2013b). Furthermore, rotifers without supplemental Se failed to boost the maximum GPx mRNA expression and activity of Atlantic cod (*G. morhua*) larvae (Penglase et al., 2010). GPx is also a significant immune effector in molluscs (Shan et al., 2011). Therefore, plasma or hepatic GPx activity is a more stringent and more convenient criterion than WG to distinguish Se status (Burk & Hill, 1993 ; Prabhu et al., 2014). The role of Se in enhancing the antioxidant defence system seems to be fundamental to biological protection from the antinutritional effects of SBM.

In this study, Se accumulation in muscle tissue was significantly higher in the OS-supplemented group. This finding is in agreement with previous studies where dietary Se concentrations in tissues were correlated to dietary Se intake (Gatlin & Wilson, 1984 ; Hilton et al., 1980 ; Lin & Shiau, 2005 ; Misra et al., 2012). However, a dose-dependent relationship of dietary OS on tissue Se accumulation was observed in the present study, with the highest Se content seen in the fish fed the highest level of OS. In addition, while other authors indicated that fish liver tissue is a better Se accumulator than muscle tissue (Ashouri et al., 2015 ; Elia et al., 2011 ; Han et al., 2011), Lorentzen et al. (1994) observed that Atlantic salmon (*S. salar*) parr fed diets supplemented with organic or inorganic Se at 1-2 mg Se kg^{-1} for 56 days accumulate Se at higher concentrations in muscle than in liver when OS was used. It is likely that the pathways of Se uptake and diffusion within aquatic animal organs depend on the form of Se used and the species examined.

Furthermore, the reduction in antioxidant capacity would be presumed to have a considerable impact on the cellular membranes of fish. Moreover, the production of ROS induces damage to biological molecules (McCord, 2000), which ultimately

results in various pathological conditions. For example, the evidence that a suboptimal Se status strengthens the risk of myopathy, a common symptom in native as well as laboratory animals, is strong in several fish species. The internal organs, muscles and liver were the site of injury when common carp (*Cyprinus carpio*) were exposed to Se-deficient diets (Wang et al., 2013). Deficiencies of Se were responsible for pancreatic tissue peroxidation in Atlantic salmon (*S. salar*) in response to reduction in antioxidant GPx activity (Bell et al., 1987). As observed in the present study, when fish were fed OS-deficient diets, necrosis and increased eosinophilia in muscle fibres occurred, causing structural changes in almost each sample examined. The affected fibres appeared to be brittle and disconnected, showing intense lesions. From this observation, skeletal muscle integrity can be used as an important marker in fish health histological-based evaluation.

The present study demonstrates that elevated dietary OS supplementation did not improve the performances of juvenile barramundi when fed high SBM diets. Instead, Se concentrations of 6.48 and 8.50 mg kg^{-1} resulted in the slightly but significantly reduced growth, haematological and antioxidant responses, although there was a survival rate exceeding 96% across all the dietary treatments. Thus juvenile barramundi performed well when fed with diets containing 3.51 to 5.48 mg Se kg^{-1}. Similarly, a dosage of 3 to 6 mg kg^{-1} of Se appears to exert a stimulatory effect on a number of biological functions in tilapia (*Oreochromis niloticus*) (Atencio et al., 2009).

Based on growth performances, the Se requirement for juvenile yellowtail kingfish (*S. lalandi*) is 5.56 mg kg^{-1} (Le & Fotedar, 2013b), much higher than that defined for grouper (*E. malabaricus*) (Lin & Shiau, 2005), cobia (*R. canadum*) (Liu et al., 2010) and loach (*P. dabryanus*) (Hao et al., 2014), being 0.7 mg Se kg^{-1}, 0.8 mg Se kg^{-1} and 0.5 mg Se kg^{-1}, respectively. Further, juvenile white sturgeon (*Acipenser transmontanus*) exposed to 20.5 and 41.7 mg Se kg^{-1} for 56 days attained 99 and 77% WG of control fish, respectively (Tashjian et al., 2006). On the other hand, Hamilton et al. (1990) observed depressed WG (only 78 and 37% of control weight) in chinook salmon (*Oncorhynchus tshawytscha*) fed Se-Met containing diets at comparable levels (18.2 and 35.4 mg Se kg^{-1}, respectively) after 60 days exposure. The discrepancies in Se requirement and toxicity may be linked to species sensitivity

to Se exposure, the source of Se and dietary components. The findings of the present study suggest that supplementing SBM-based diets with OS above 3 g kg^{-1} is superfluous. However, Fontagne-Dicharry et al. (2015) emphasised that feeding rainbow trout (*O. mykiss*) with PP-based diets lacking Se supplementation could be harmful to the antioxidant capacity of fish. Therefore, addition of Se to PP-based diets is an important issue.

5.5. Summary

In conclusion, dietary OS supplementation at an appropriate level improved growth, antioxidant activity, accumulation, and histological performances. Therefore, a dietary Se requirement of 3.5-4.5 mg Se kg^{-1} is proposed to be the optimum level for juvenile barramundi fed high SBM diets if growth and physiological health is the aim. In addition, erythrocyte GPx is a beneficial indicator of Se deficiency in juvenile barramundi. The findings of the present study should reinforce the necessity to conduct a study to investigate the effect of a more refined SBM product along with OS supplementation in the practical diets for juvenile barramundi.

CHAPTER 6: Effects of organic selenium supplementation on growth, glutathione peroxidase activity and histopathology in juvenile barramundi (*Lates calcarifer* Bloch 1970) fed high lupin meal-based diets

This research is published in Aquaculture
Volume 457 (2016): 15-23

6.1. Introduction

Fishmeal (FM), the primary protein source in aquaculture feeds, are fabricated from either whole fishes, fish cut-offs or fish processing by-products (Shepherd & Jackson, 2013). Demand for FM increases as aquaculture production intensifies, resulting in luxurious prices for the commodity (OECD/FAO, 2014). In addition, there is also concern over the continuity of FM supply along with the finite nature of wild fisheries (Delgado et al., 2003). These economic and ecological sustainability issues are putting more pressures on the aquaculture industry to lower the levels of FM in aquaculture feeds (Gallagher, 1994 ; Hardy, 2010 ; Hua & Bureau, 2012). Nevertheless, the sustainability of the aquaculture industry is and will be fundamentally relying on the reduction of FM in feed composition (Bostock et al., 2010 ; Bulbul et al., 2013) or the shift from FM to non-FM as the major protein sources used in aquaculture feeds (Hardy, 2010 ; Huntington & Hasan, 2009). Therefore, numerous studies embodying a wide variety of ingredients, feed formulations, and experimental system have been undertaken to investigate the inclusion of non-FM dietary component derived from plant protein (PP) sources, which are considered to be more cost-effective and more ecologically-friendly.

Among PP sources, lupin meal (LM, *Lupinus angustifolius*) has received considerable attention as a potential alternative to FM due to its comparatively balanced nutritional profile, desirable palatability, high digestibility, cheaper price and reliable supply (Gatlin et al., 2007). The LM obtained after dehulling process is a favourable protein source with protein content ranging between 350 g kg^{-1} and 500 g kg^{-1} dry matter (DM) (Drew et al., 2007). Several studies have shown considerable success in low replacement of FM with LM (≤30%) in diets for a variety of fish species (Glencross et al., 2004b ; Glencross & Hawkins, 2004 ; Glencross et al., 2008 ; Omnes et al., 2015 ; Pereira & Oliva-Teles, 2004 ; Refstie et al., 2006b ;

Zhang et al., 2012a ; Zhang et al., 2012b). However, a number of studies show that partial or high replacement of FM with LM (≥50%) is conceivable at least in rainbow trout (Borquez et al., 2011 ; Burel et al., 1998 ; Farhangi & Carter, 2007 ; Glencross et al., 2003b ; Glencross et al., 2004b). Reasons for discrepancy among aquaculture nutritionists on the utilisation of LM as a protein source for fish might be associated with a number of factors including product quality, treatment and inclusion levels of LM, feed formulation, culture condition, fish size and variations in fish species.

For carnivorous species, problems occur when FM is highly or fully replaced with PP ingredients in the diet. Those include reduced fish performance and health caused by poor palatability, AA deficiency, lower nutrient digestibility, decreased energy content as well as the manifestation of particular compounds in plants that are unfavourable to fish, identified as anti-nutritional factors (ANF) (Bonaldo et al., 2011 ; Farhangi & Carter, 2001 ; Francis et al., 2001 ; Krogdahl et al., 2010 ; NRC, 2011). Moreover, certain ANF such as phytic acid may reduce the bioavailability of minerals such as copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), manganese (Mn), iron (Fe), magnesium (Mg), calcium (Ca) and selenium (Se) (Connelly, 2011). The complexity of mineral chelation and subsequent deficiencies can impair growth and health of fish fed diets containing high PP ingredients. Therefore, the use of mineral-derived feed additive in the low FM diet is an alternative approach to diminish the adverse effects of PP sources and may help improve fish growth and health performance.

Se is a trace mineral essential for fish cellular metabolism. However, it becomes poisonous for aquatic organisms at high concentrations. Se serves as an integral structural element of the active core of glutathione peroxidase (GPx) enzymes in red blood cells (RBC) (Rotruck et al., 1973). Beneficial effects of supplementing diets of a variety of fish species with Se additives have been well documented in previous studies (Arshad et al., 2011 ; Bell et al., 1987 ; Bell et al., 1986 ; Elia et al., 2011 ; Gatlin & Wilson, 1984 ; Hardy et al., 2010 ; Hilton et al., 1980 ; Jaramillo Jr et al., 2009 ; Kucukbay et al., 2009 ; Le & Fotedar, 2013b ; Le & Fotedar, 2014b ; Le et al., 2014b ; Lin & Shiau, 2005 ; Liu et al., 2010 ; Lorentzen et al., 1994 ; Rider et al., 2009). However, none of those studies utilised PP ingredients as the protein

source in the diet, and, instead, FM were used as the main input for dietary protein. The importance of dietary Se in PP-derived ingredients such as LM remains a lacuna in fish nutrition studies (Prabhu et al., 2014) including those applied for marine carnivorous finfish species.

Barramundi (*Lates calcarifer*), also known as Asian seabass, is a carnivorous fish which is widely distributed throughout the Asia-Pacific region. The species has been an economically important species in Australia and Asian countries (Paul et al., 2013). One advantage to barramundi aquaculture is that, due to their osmoregulatory capacity, this species can be reared in both seawater and freshwater environments. Although barramundi are fed with commercial diets in Australia, feeding with trash fish is very common in several Asian countries (Job, 2011 ; Rimmer & John Russell, 1998 ; Tantikitti et al., 2005). Nutritional requirements of barramundi have been extensively studied; however, less attention has been paid to trace element nutrition of the species. Therefore, this study was carried out to investigate the effect of OS supplementation in barramundi diets containing LM as the major dietary protein source. Growth performance, feed utilisation, blood chemistry and histopathology were particularly examined.

6.2. Materials and methods

6.2.1. Diets and experimental design

Three isonitrogenous (48.8% crude protein, CP) and isoenergetic (20.6 MJ kg^{-1} gross energy, GE) experimental diets were prepared and formulated, as LM_0, LM_{25}, and LM_{75}. Diets were supplemented with 0 and 2 g OS kg^{-1} dry matter (DM). Thus, there were two control diets in the feeding experiment: FM-based diet without OS supplement (LM_0) and with OS supplement (LM_{0+OS}). FM, LM, casein and gluten were used as protein sources in the diets. Formulation and proximate composition of the experimental diets are presented in Table 6.1. Water (50 g kg^{-1}) was added prior to pelleting. All ingredients were ground to pass through a 1-mm mesh screen, pelleted in a mixer, crumbled to the desired size, air-dried, and stored at 4°C until feeding. Cr_2O_3 was included in all diets at 0.5% as an inert, indigestible marker to determine apparent digestibility coefficient (ADC) of protein. AA profiles of the experimental diets are shown in Table 6.2.

Table 6.1. Formulation and proximate composition of the experimental diets

Ingredients[a] (g kg^{-1} DM)	Diets					
	LM_0	LM_{0+OS}	LM_{25}	LM_{25+OS}	LM_{75}	LM_{75+OS}
Fishmeal	460	460	340	340	150	150
Lupin kernel meal[b]	-	-	185	185	510	510
Wheat gluten	100	100	100	100	100	100
Wheat flour	80	80	40	40	-	-
Casein	120	120	120	120	120	120
Fish oil	100	100	100	100	80	80
Wheat starch	65	63	50	53	15	13
Cellulose	50	48	40	38	-	-
Se-free premix[c]	20	20	20	20	20	20
Organic selenium[d]	-	2	-	2	-	2
Chromic oxide	5	5	5	5	5	5
Proximate content (%)						
Dry matter	89.20	89.35	90.15	90.25	90.18	90.23
Ash	8.13	8.37	5.82	5.88	5.35	5.61
Protein	49.33	49.50	48.75	48.81	48.25	48.16
Lipid	15.41	15.40	15.07	15.05	14.52	14.55
Gross energy (MJ/kg)	20.59	20.41	20.71	20.45	20.75	20.69
Se (mg kg^{-1})	1.48	3.45	1.35	3.38	1.21	3.25

[a] Supplied by Specialty Feeds, Perth, WA, Australia, except for Sel-Plex and chromic oxide, obtained from AllTech, Lexington, Kentucky, USA and Thermo Fisher Scientific, Scoresby, Vic, Australia, respectively.

[b] Australian sweet lupin, *Lupinus angustifolius*

[c] Contains the following (as g kg^{-1} of premix): iron, 10; copper, 1.5; iodine, 0.15; manganese, 9.5; zinc, 25; vitamin A retinol, 100 IU; vitamin D3, 100 IU; vitamin E, 6.25; vitamin K, 1.6; vitamin B1, 1; vitamin B2, 2.5; niacin, 20; vitamin B6, 1.5; calcium, 5.5; biotin, 0.1; folic acid, 0.4; inositol, 60; vitamin B12, 0.002; choline, 150; ethoxyquin, 0.125.

[d] Sel-Plex®

Table 6.2. Hydrolysed amino acid composition of FM, LM, wheat gluten and casein (g 100 g^{-1} protein)

Amino acid	FM	LM	Wheat gluten	Casein
Essential				
Arginine	4.35	4.67	1.50	3.32
Histidine	2.09	1.11	0.96	2.73
Isoleucine	3.04	1.80	2.35	4.70
Leucine	5.17	2.90	9.75	8.70
Lysine	4.77	1.84	0.80	7.46
Methionine	1.86	0.31	1.05	2.19
Phenylalanine	2.87	1.78	3.47	4.67
Threonine	3.19	1.58	1.76	3.71
Valine	3.26	1.70	2.58	5.75
Non-essential				
Alanine	4.42	1.52	NA	2.56
Aspartic acid	6.20	4.15	NA	6.24
Glutamic acid	8.25	8.84	NA	18.60
Glycine	4.73	1.75	NA	2.38
Proline	3.81	2.12	NA	8.80
Serine	3.05	2.14	NA	5.36
Taurine	0.31	0.00	NA	NA

NA: not analysed

6.2.2. Fish and experimental conditions

Three hundred and sixty healthy barramundi juveniles of average weight 5.38 ± 0.16 g (mean ± SE) were supplied by the Australian Centre for Applied Aquaculture Research (Fremantle, Australia). Prior to the feeding trial, fish were acclimated and fed with a commercial diet twice daily for 2 weeks until fully acclimated to the rearing conditions. At the commencement of feeding trial, eighteen groups with 20 fish each were bulk weighed and then randomly stocked into 18 tanks. Each experimental diet was triplicated.

The feeding trial was conducted at the Curtin Aquatic Research Laboratory (Technology Park Bentley, Australia). The experimental system consisted of 18 circular, 300-L fibreglass tanks. Each tank received recirculated water from an

external biofilter (Fluval 406, Hagen, Italy) at 10 L min^{-1}. All experimental tanks were supplied with constant aeration and pure oxygen (compressed oxygen, BOC, Perth, WA, Australia).

The feeding trial lasted for 60 days, during which fish were hand-fed with the experimental diets to satiation twice daily at 0900 h and 1500 h. Throughout the experiment, the water quality parameters were maintained at temperature 27 – 29ºC, dissolved oxygen >5 mg/L, and salinity 32-34 ppt. Dead fish were weighed and recorded for adjusting the calculation of feed conversion ratio (FCR) and survival. Fish handling procedures, care, and facilities complied with the guidelines of the Animal Ethics Committee of Curtin University and followed the Australian Code of Practice for the care and use of animals for scientific purposes.

6.2.3. Protein digestibility

To investigate the effect of dietary treatment on protein digestibility, faecal matter was collected immediately prior to the morning feeding by stripping techniques (Austreng, 1978) one week before the end of the feeding experiment. Faecal collections from individuals were pooled by tank and quickly stored at –20ºC. Prior to analysis, the faecal samples were dried to constant weight at 105 ºC. ADC of protein was measured using the indirect method (Cr_2O_3), as suggested by Cho *et al*. (1982).

6.2.4. Sampling and analytical methods

At the measurement of the terminal body weight, all fish were starved for 24 h prior to final sampling to achieve a basic metabolite state. The fish were then anaesthetised with tricaine methanesulfonate (MS-222, Sigma-Aldrich, Castle Hill, NSW, Australia) at 100 mg L^{-1} and weighed individually. Blood samples from three fish each tank were then withdrawn by caudal vein puncture with a 1-mL plastic syringe. The extracted blood was transferred to a heparinised tube for haematology. Haematocrit and leucocrit were determined by centrifugation of capillary glass tubes, according to the method of McLeay & Gordon (1977). An Hb kit (Randox Laboratories, Antrim, United Kingdom) was used to measure the haemoglobin (Hb) content. Erythrocyte (red blood cell) glutathione peroxidase (GPx) activity was

quantitatively assayed using the Randox Laboratories test combination (Ransel, Antrim, United Kingdom).

The proximal compositions of the diet and faecal samples were determined based on Association of Official Analytical Chemists procedures (AOAC, 1990). Dry matter was determined by oven drying to constant weight at 105ºC; crude ash by combustion at 550 ºC; crude protein content (N × 6.25) by the Kjeldahl digestion method; crude lipid content by the Soxhlet technique; and gross energy content by an IKA oxygen bomb calorimeter (Heitersheim, Germany). Amino acid (AA) content of the diets was determined after samples were hydrolysed in HCl (Barkholt & Jensen, 1989 ; Rayner, 1985). Analyses were performed on an Agilent 1100 series high-performance liquid chromatography (HPLC, Agilent Technologies, Germany) system using conditions similar to those described by Gratzfeld-Huesgen (1998).

6.2.5. Se determination

Se was analysed at the Intertek Genalysis Laboratory (Perth, Australia) using inductively coupled plasma-mass spectrometry instrument (ICP-MS, 7500 series, Agilent Technologies, Australia). The total Se in diet and muscle tissue samples was determined using an aqua regia digestion with reduction precipitation. 0.5 g sample was dissolved in aqua regia, filtered, and the selenium reduced to the zero oxidation state where it precipitated. The precipitate was collected using filtration, dissolved in acid and read on an ICP-MS. A mass of 5 g was catch-weighed in a beaker. Nitric acid (HNO_3) and hydrochloric acid (HCl) were added sequentially to make aqua regia. The excess acid was boiled off and the digested sample was leached in HCl. The sample was filtered, the residue and beaker were thoroughly washed, and the filtrate was collected.

The selenium was precipitated using a Cu solution in a mixture of citric and ascorbic acids at a low temperature. The selenium was filtered off and dissolved in nitric acid. HCl and deionised water were added and the solution was mixed and read on a calibrated ICP-MS. Selected certified reference material (CRM) for the analysis was OREAS 97.01, which was prepared from OREAS 97 (Ore Research and Exploration Pty Ltd, Victoria, Australia) by diluting this 100× in high purity silica. The secondary CRM OREAS 97.01 had an aqua regia extractable value of 0.673 ± 0.063

mg Se kg^{-1}. Recovery of the CRM was 98-99% and the limit of detection (LOD) was 0.01 mg Se kg^{-1}. Se concentration was reported as dry weight.

6.2.6. Histopathology

At the end of the trial, segments of muscle, liver, kidney, spleen and intestine from three fish each tank were fixed in 10% buffered formalin, dehydrated in ethanol before equilibration in xylene and embedding in paraffin wax. Sections of approximately 5 µm were cut and stained with haematoxylin and eosin for histological observation under an Olympus BX40F4 light microscope. Light microscopy samples were prepared according to standard histological techniques (Luna, 1968).

6.2.7. Calculation

Growth and feeding performances were measured using the calculated parameters below:

$$\text{Specific growth rate (SGR, \% day}^{-1}) = 100 \times \left[\frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{days}}\right]$$

$$\text{Weight gain (\%)} = 100 \times \left[\frac{\text{final weight-initial weight}}{\text{initial weight}}\right]$$

$$\text{Feed intake (FI, g fish}^{-1}\text{ days}^{-1}) = \left[\frac{\text{diet given} - \text{remaining diet recovered}}{\text{number of fish}}\right] / \text{days}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake}}{\text{weight gain}}$$

$$\text{Survival (S, \%)} = 100 \times \left[\frac{\text{final number of fish}}{\text{initial number of fish}}\right]$$

$$\text{ADC (\%)} = 100 - \left[(100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\text{ protein in faeces}}{\%\text{ protein in diet}})\right]$$

$$\text{Thermal growth coefficient (TGC)} = \left[\frac{(\text{final weight}^{1/3} - \text{initial weight}^{1/3})}{\text{temperature℃} \times \text{days}}\right] \times 1000$$

6.2.8. Statistical analysis

All data regarding the effects of LM level, Se level and their interactions on growth, feeding, haematological and enzymatic GPx responses, as well as muscle Se content were subjected to two-way analysis of variance (ANOVA). Assumptions of

homogeneity of variances were checked using Levene's equal variance test. When a significant interaction of the two factors was detected, the main effects were not further considered, and the variable was analysed by one-way ANOVA followed by Duncan test to inspect all differences among the dietary treatments. When there was no significant interaction between the two factors, then the main effect was examined for both factors using Duncan test to check all differences among the dietary treatments. All differences were considered significant when P value < 0.05. All statistical analyses were performed using IBM SPSS software (version 22, Australia).

6.3. Results

6.3.1. Growth, survival and protein ADC

Data for FW, SGR, FI, WG, FCR, ADC-P, Survival and TGC are presented in Table 6.3. All experimental diets were voluntarily ingested by juvenile barramundi over the course of the feeding experiment and, thus, no significant differences were observed in FI. OS supplementation levels influenced FW, SGR and WG. Fish fed diets containing OS had higher FW, SGR and WG compared with those fed diets lacking OS supplementation ($P < 0.05$). LM inclusion levels and OS supplementation levels affected ADC of protein. Fish fed diets containing OS supplement attained higher ADC-P in comparison with those fed OS-deficient diets. In addition, irrespective of OS supplementation level, ADC of protein decreased as dietary LM increased. Survival exceeded 98% and did not differ significantly among dietary treatments. Meanwhile, LM inclusion levels, OS supplementation levels, or the interaction of these factors, did not affect TGC.

6.3.2. Blood physiology and muscle Se level

There was no synergistic effect between OS supplementation and LM inclusion levels on the haematocrit, leucocrit, GPx activity and muscle Se concentration (Table 4). No significant effect was observed on haematocrit ($P > 0.05$). However, increased leucocrit was found in fish fed OS-containing diets. OS supplementation levels significantly affected GPx activity. GPx activity of fish in OS-supplemented group ranged from 197 to 205 (U^{-1} g Hb), higher than those without OS supplementation. Muscle Se concentration was influenced by both LM inclusion and OS

supplementation levels and muscle Se contents were significantly affected by both LM inclusion and OS supplementation levels ($P < 0.05$). In addition, muscle Se concentration was related to the Se level in the diets (Figure 6.1).

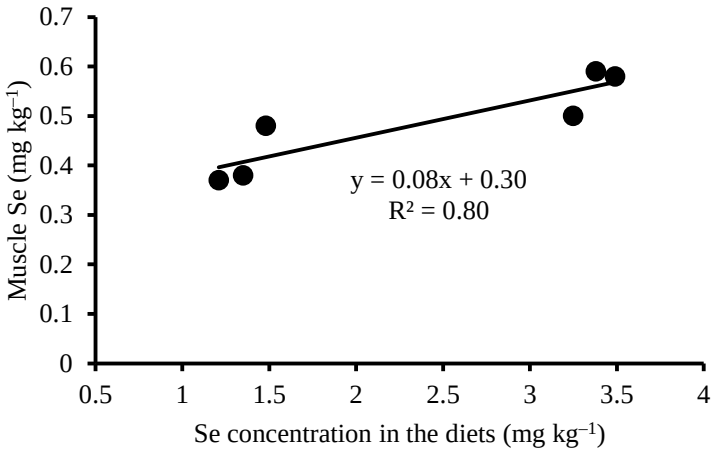

Figure 6.1. Relationship between Se concentration in the diets and muscle Se level of juvenile barramundi after 60 days feeding trial.

Table 6.3. Performance of juvenile barramundi fed different LM levels with and without OS supplementation for 60 days[1]

Parameters	0 g kg^{-1} OS			2 g kg^{-1} OS			Two-way ANOVA (*P* value)		
	LM_0	LM_{25}	LM_{75}	LM_0	LM_{25}	LM_{75}	LM	OS	LM × OS
FW	31.6 ± 0.97	30.2 ± 0.82	29.8 ± 1.15	34.4 ± 0.78	35.4 ± 1.61	33.3 ± 0.48	0.148	0.001	0.309
SGR	3.17 ± 0.03	3.13 ± 0.03	3.04 ± 0.31	3.30 ± 0.08	3.32 ± 0.05	3.26 ± 0.23	0.298	0.002	0.708
WG	490.3 ± 4.59	477.5 ± 5.88	449.5 ± 3.51	533.6 ± 5.22	543.7 ± 1.97	522 ± 2.82	0.336	0.002	0.842
FI	0.63 ± 0.01	0.64 ± 0.02	0.61 ± 0.01	0.64 ± 0.03	0.62 ± 0.01	0.62 ± 0.03	0.133	0.869	0.265
FCR	1.26 ± 0.03	1.24 ± 0.02	1.30 ± 0.02	1.23 ± 0.04	1.22 ± 0.01	1.29 ± 0.03	0.507	0.052	0.723
ADC-P	90.4 ± 1.23	92.8 ± 0.75	94.3 ± 0.80	93.4 ± 1.17	94.8 ± 0.67	94.7 ± 0.66	0.038	0.019	0.068
S	98.3 ± 1.67	96.7 ± 1.67	98.3 ± 1.67	100 ± 0.00	98.3 ± 1.67	98.3 ± 1.67	0.695	0.493	0.884
TGC	0.851 ± 0.04	0.844 ± 0.02	0.802 ± 0.02	0.840 ± 0.07	0.886 ± 0.05	0.837 ± 0.06	0.213	0.279	0.478

LM0, LM25, LM75 (FM protein replaced by LM protein with 0%, 25% and 75%, respectively).
FW (final weight, g); SGR (specific growth rate, % body weight day^{-1}); FI (feed intake, g $fish^{-1}$ day^{-1}); WG (weight gain, %); FCR (feed conversion ratio); ADC-P (apparent digestibility coefficient – protein, %); S (survival, %); TGC (thermal growth coefficient, day 1 – 60; 28.5ºC)
Values in the same row without a common superscript are significantly different ($P < 0.05$)

Table 6.4. Haematocrit, leucocrit, GPx activities and muscle Se concentration of barramundi fed different LM levels with and without OS supplementation for 60 days[1]

Parameters	0 g kg^{-1} OS			2 g kg^{-1} OS			Two-way ANOVA (*P* value)		
	LM_0	LM_{25}	LM_{75}	LM_0	LM_{25}	LM_{75}	LM	OS	LM × OS
Haematocrit	33.30 ± 0.58	32.76 ± 0.52	33.27 ± 0.48	32.30 ± 0.32	34.73 ± 0.27	33.07 ± 0.61	0.608	0.752	0.284
Leucocrit	1.14 ± 0.02	1.05 ± 0.03	1.01 ± 0.01	1.42 ± 0.03	1.46 ± 0.03	1.18 ± 0.02	0.149	0.003	0.479
GPx	195.7 ± 5.18	194.4 ± 3.37	193.3 ± 5.93	205.3 ± 1.66	199.7 ± 2.05	197 ± 1.89	0.185	0.018	0.590
Muscle Se	0.48 ± 0.02	0.38 ± 0.02	0.37 ± 0.03	0.58 ± 0.01	0.59 ± 0.01	0.50 ± 0.03	0.005	0.000	0.300

LM0, LM25, LM75 (FM protein replaced by LM protein with 0%, 25% and 75%, respectively).
Haematocrit (%); Leucocrit (%), GPx (U/g Hb); Muscle Se (μg/g)
Values in the same row without a common superscript are significantly different ($P < 0.05$).

6.3.3. Histopathological evaluation

As observed in the tissue of fish fed diets supplemented with OS, the normal structure of skeletal muscle in cross-section was characterised by rounded, densely packed, and uniformly identical muscle fibres (Figure 6.2a and c). However, severe histopathological alterations were observed in muscle tissue of fish fed LM_{25} and LM_{75} diets. The skeletal muscle exhibited signs typical of nutritional muscular dystrophy attributable to Se deficiency, including falsification, disconnection, and longitudinal rupture of muscle fibres (Figure 6.2b and d). Also, LM diets lacking OS supplementation caused notable morphological changes in the liver of fish, which were characterised by variations in hepatocyte vacuolation, with an increased amount of lipid droplets found in the liver of fish fed the LM_{75} diets (Figure 6.3). However, all tissues of the gastrointestinal tract were histologically normal, as were the kidney and spleen.

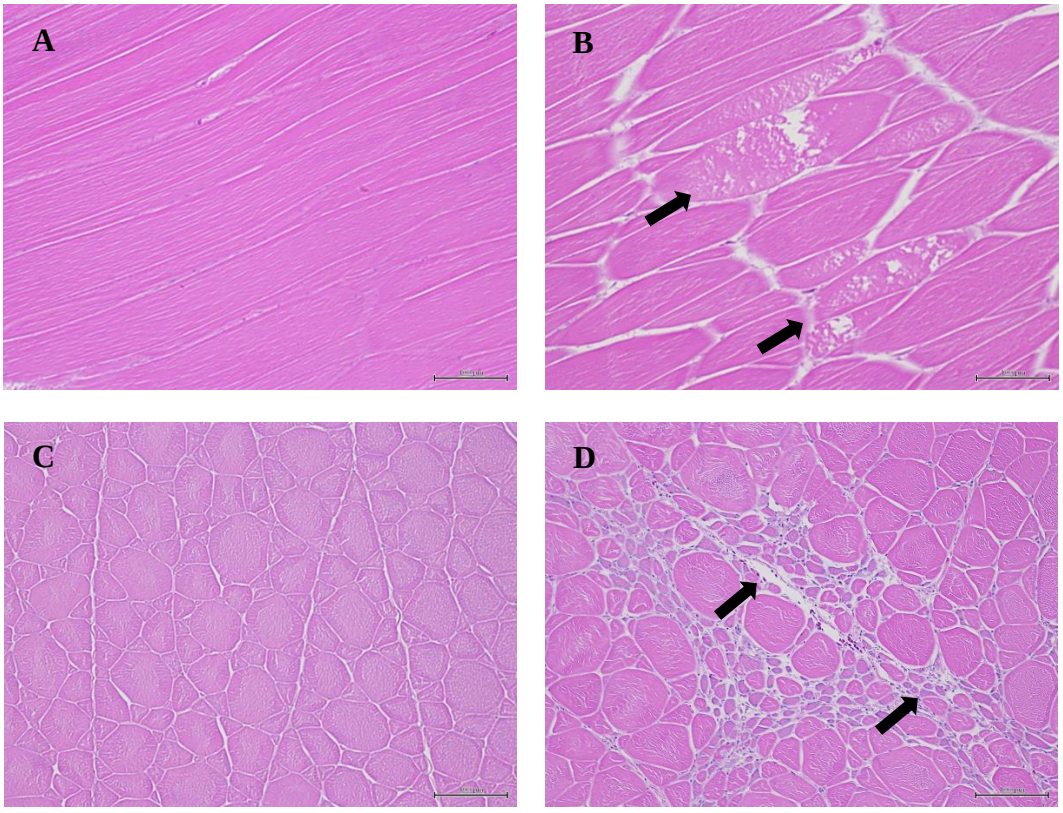

Figure 6.2. Longitudinal and transverse-section of the muscle of barramundi showing normal histological structures (A,C), and Se deficiency-induced myopathy (B,D). Note severe muscle degeneration and hypercontraction of the surrounding muscular fibres (arrows). Scale bar = 100 μm.

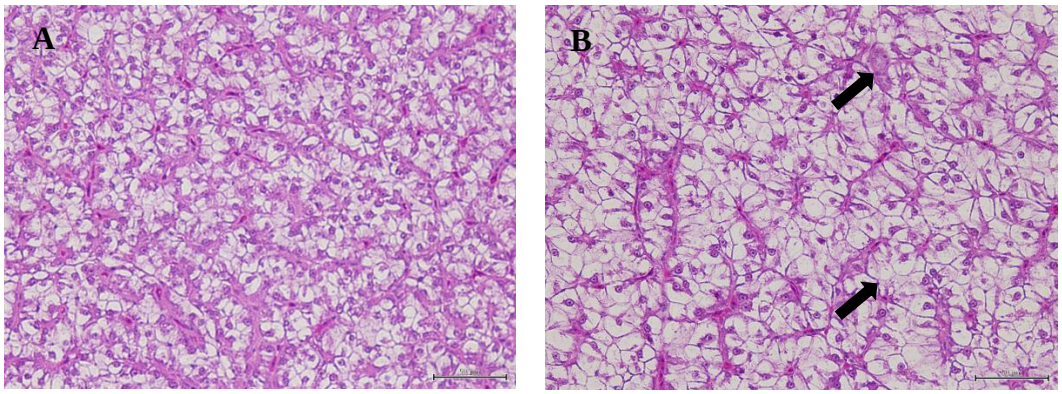

Figure 6.3. Sections of liver of barramundi showing normal liver which contain predominantly glycogen vacuoles (A), and fatty liver (B). Note the extension the hepatocytes and the generation of apparent hepatic steatosis, with intense vacuoles in the hepatocytes resemble lipids (arrows). Scale bar = 50 μm.

6.4. Discussion

The inclusion level of FM in compounded aquaculture feed has been substantially reduced, and PP materials have been, and will possibly remain, the foremost alternative when substituting FM in fish diets. In recent years, aquaculture feed development has been directed towards further reduction of FM as the main protein source in fish feeds without hampering growth performance and nutrient utilisation (Olsen & Hasan, 2012). To achieve this, nutritional strategies should include not only finding other PP sources as alternatives to FM but also improving the quality of the already existing PP materials through innovative approaches, such as proper raw material processing and nutrient enrichment. For instance, Krogdahl et al. (2010) suggested that the presence of phytic acid ANF in PP ingredients might be counteracted by mineral supplementation. Indeed, effects of Se supplementation on performance of fish fed PP-based protein sources remain unstudied (Prabhu et al., 2014).

One of the undesirable features of PP-based diets has been related to low FI. Several authors have reported the suppression of FI with high proportion of PP sources (Alexis, 1990 ; Espe et al., 2006 ; Gomes et al., 1995 ; Refstie et al., 1998 ; Torstensen et al., 2008). In the case of LM, a reduction in FI was attributable to alkaloid content (de la Higuera et al., 1988), high-fibre substance (Bangoula et al., 1993), AA deficiency (Jobling et al., 2007) and the presence of ANF (Francis et al., 2001) in the diets. The effects on FI are commonly manifested in nutrient intake and

thus growth and health performance. In the current experiment, the fish promptly accepted all experimental diets, and the FI was not then an inducer in the resulting growth indices. This might show that the level of detractive compounds in PP-based diets employed in the current study was inadequate to deteriorate FI. Moreover, Australian sweet lupin *L. angustifolius*, used in the present study, is minimal in alkaloid contents, and thus palatability problems due to elevated alkaloids, as reported by Glencross et al. (2006a), were not found.

To our knowledge, this is the first study to have investigated the effects of OS as feed supplement in LM diets on the growth, feed utilisation, blood physiology and histopathology of marine carnivorous finfish species. In the present experiment, the FW, SGR and WG of juvenile barramundi fed high LM diets were significantly lower than those fed OS-supplemented LM diets. The finding that high PP inclusion level (≥ 50%) resulted in unfavourable growth performance agrees with the results reported for similar species (Tantikitti et al., 2005) and other marine carnivorous finfish species (Chou et al., 2004 ; Farhangi & Carter, 2001 ; Glencross et al., 2004b ; Hernández et al., 2007 ; Peng et al., 2013 ; Wang et al., 2006b ; Zhou et al., 2005). In contrast, several authors have reported that 50–70% LM can be incorporated in the marine carnivorous finfish diets (Borquez et al., 2011 ; Burel et al., 1998). In addition, with reference to growth performance, Gallagher (1994) found that soybean meal (SBM) could replace 75% FM protein in hybrid striped bass (*Morone saxatilis*) feeds. The depression in overall growth indicators in fish fed OS-deficient diets may be associated with the existence of phytic acid (PA) in LM.

PA, or phytate in salt form, is the primary phosphorus (P) storage compound in seeds, typically representing 50–80% of the total P in plant meals and providing around 1.5% to the meal dry weight (Kumar et al., 2012). It has been a general consensus that most finfish and land monogastric animals cannot utilise the P bound in PA since they lack the enzyme (phytase) that is needed to mortify phytate (Robaina et al., 1995). Inclusion of phytate (18 g kg^{-1}) in diets for Atlantic salmon (*Salmo salar*) caused a substantial reduction in growth, feed utilisation and mineral bioavailability (Storebakken et al., 1998). Retarded growth and reduced mineral absorption was obvious in striped bass (*M. saxatilis*) when served diets containing high phytate content (Papatryphon et al., 1999). Furthermore, in the presence of high

phytate with low Zn diet, feed utilisation and growth of Chinook salmon (*Oncorhynchus tshawytscha*) were depressed, resulting in high mortality and irregularities in pyloric caecal formation (Richardson et al., 1985).

However, the results of the present experiment indicate that, when supplemented with 2 mg kg^{-1} OS, 75% FM protein could be replaced with LM protein, without triggering a considerable adverse implication on growth, feed utilisation, blood chemistry or histology of juvenile barramundi. This is in accordance with results from our earlier studies (unpublished), in which supplementation of 2 mg kg^{-1} OS in high SBM-based diets also resulted in improved fish performances; FW and SGR were slightly lower when barramundi were fed high SBM diets, but similar if not better growth performances were attained in fermented SBM fed to barramundi. A synergistic effect between dietary vitamin E and Se concentration in the diets reportedly modified the dietary Se requirement (Jaramillo Jr et al., 2009 ; Le et al., 2014b). However, the required vitamin E level of barramundi was assumed to be satisfied in the present experiment with the supplementation of 130 mg α-tocopherol kg^{-1} DM.

Table 6.5 shows a range of FM-based studies confirming the significance of dietary Se in various fish species. However, only a few adhere to European Union (EU) feed legislation that has set an upper limit of 0.5 mg Se kg^{-1} for animal including fish (EU, 2015). Indeed, in most finfish species, dietary Se requirement of fish varies with species, age, the form of Se ingested, vitamin E level of the diet and pathway and duration of exposure to Se (NRC, 2011). Further, high replacement level of FM with PP ingredients in aquaculture feeds might affect the supply of minerals including P, Zn and Se (Prabhu et al., 2014). In the present study, although the dietary concentrations of Se were relatively high (1.58–3.11 mg Se kg^{-1}), the inhibitory effect of PA could have depressed the absorption of Se, and therefore elevated Se levels were required in the diets for juvenile barramundi.

Table 6.5. Dietary Se source, level and requirement (mg kg^{-1}) in various fish species

No	Species	Se Source	Se level (mg kg^{-1})	Requirement (mg kg^{-1})	Reference
1	Atlantic salmon (*S. salar*)	Se-Met	2.1–3.1	<2.1	Lorentzen et al. (1994)
2	Beluga sturgeon (*Huso huso*)	Se-Met	1.26–20.3	11.56	Arshad et al. (2011)
3	Channel catfish (*Ictalurus punctatus*)	Na_2SeO_3	0.06–5.0	0.25	Gatlin and Wilson (1984)
4	Channel catfish (*I. punctatus*)	Se-Met	0.02–0.4	0.12	Wang and Lovell (1997)
5	Cobia (*Rachycentron canadum*)	Se-Met	0.21–1.4	0.8	Liu et al. (2010)
6	Common carp (Cyprinus carpio)	Nano-Se	0.43–2.51	1.46	Ashouri et al. (2015)
7	Cutthroat trout (*Oncorhynchus clarkii*)	Se-Met	1.2–11.2	9.2	Hardy et al. (2010)
8	Gibel carp (*Carassius auratus gibelio*)	Se-Met	0.34–5.13	1.18	Han et al. (2011)
9	Grouper (*Epinephelus malabaricus*)	Se-Met	0.17–4.0	0.7	Lin and Shiau (2005)
10	Hybrid striped bass (*Morone chrysops* × *M. saxatilis*)	Na_2SeO_3	1.19–21.23	1.19	Jaramillo Jr et al. (2009)
11	Hybrid striped bass (*M. chrysops* × *M. saxatilis*)	Na_2SeO_3	1.22–4.42	0.4	Cotter et al. (2008)
12	Largemouth bass (*Micropterus salmoide*)	Na_2SeO_3	0.97–2.06	1.60–1.85	Zhu et al. (2012)
13	Yellowtail kingfish (*Seriola lalandi*)	Organic (Sel-Plex®)	4.86–6.38	5.56	Le and Fotedar (2013b)

The OS used in this experiment was in the form of selenised yeast (Sel-Plex®, Alltech Inc., Lexington, Kentucky, USA) that contains a mixture of selenoamino acids with Se-Met representing more than 50% of the total Se (Lyons et al., 2007). Trace element amino acid chelates (AAC) have been widely used in animal nutrition. They have shown to improve mineral bioavailability and affect growth and tissue mineral deposition in several terrestrial and aquatic animals (Sarker et al., 2007 ; Satoh, 2007). Owing to protection of the structural alignment of chelates, it is likely that, when ingested as AAC, there is a minor chance of the complexing action of phytate on mineral cations occurring (Apines-Amar et al., 2004). Interestingly, this may explain, indirectly, the increased ADC-P observed in fish fed the OS-containing LM diets, as revealed in the present experiment. As OS was supplemented in the diets, high absorption of AA-chelated Se into the mucosal tissues may promote an increased source of essential trace element that was later utilised as a co-factor in the production of hydrolytic enzymes, such as gastro-intestinal (GI) GPx, in the mucosal tissues. Moreover, GI-GPx is the most influential selenoprotein antioxidant in preserving the intestinal mucosal integrity (Lindh, 2013). A substantial quantity of digestive enzymes in the gut would presumably instigate the enhancement of feed digestion.

Nevertheless, regardless of OS supplementation, LM inclusion levels in the diets influenced the ADC-P in the present experiment. The ADC-P was shown to increase with increasing LM inclusion levels. At 75% LM protein inclusion level, the ADC-P was around 94.3–94.7%, significantly higher than FM-based diets (90.4–93.4%). This finding was in agreement with the previous studies using similar species (Glencross, 2006 ; Glencross, 2011 ; Tabrett et al., 2012), which suggested that LM protein was typically better digested than animal-derived protein. In addition, Boonyaratpalin et al. (1998) found that, when fed to barramundi, the ADC-P of SBM products (94% in average) were higher than FM (92%), with the exception of raw SBM (73%). High ADC-P of LM-based diets has also been reported in diets for rainbow trout (*Oncorhynchus mykiss*) (Borquez et al., 2011 ; Burel et al., 1998 ; Glencross et al., 2007b ; Glencross et al., 2010b). In comparison, the ADC-P of LM ingredients was reported to be identical to that of FM in diets for rainbow trout (*O. mykiss*) (de la Higuera et al., 1988) and gilthead seabream (*Sparus auruta*) (Robaina

et al., 1995). Perhaps differences in ADC-P are related to variations in product quality, diet composition and species being examined.

FM-based diets, in general, represent a major source of minerals and trace elements to satisfy the nutritional prerequisites of fish. However, FM-based diets without Se supplement have been reported to generate reduced antioxidant capacity when fed to several fish species (Kucukbay et al., 2009 ; Le & Fotedar, 2014b ; Liu et al., 2010 ; Pacini et al., 2013 ; Rider et al., 2009). The most important biological significance of Se is its antioxidant capacity mediated through GPx that provides cellular protection against toxic peroxides (Arthur et al., 2003 ; Watanabe et al., 1997). Thus, insufficient intake of Se induces low GPx activity (Dhur et al., 1990). However, based on molecular genetics, Penglase et al. (2014) observed that whole body GPx activity and expression was at the minimum at maximum growth rate in zebrafish (*Danio rerio*), thus the relationship between Se requirement and GPx activity is not straightforward in fish.

Because muscle tissue is a strong Se accumulator, muscle Se level has been associated with GPx activity in a variety of fish species (Bell et al., 1985 ; Han et al., 2011 ; Hilton et al., 1980 ; Kucukbay et al., 2009 ; Le & Fotedar, 2014a ; Wang et al., 2007 ; Zhou et al., 2009 ; Zhu et al., 2012). However, there was a dose-dependent relationship between muscle Se concentration and dietary Se level (Le & Fotedar, 2014c). Further, Fontagne-Dicharry et al. (2015) investigated the effects of dietary Se enrichment on the antioxidant status of rainbow trout (*O. mykiss*) fed diets containing 75% PP mixtures (wheat gluten, corn gluten meal, soybean protein concentrate, soya meal, rapeseed meal, white LM and dehulled pea meal). This is so far the only information available regarding the impacts of Se inclusion in PP-based diets. While there was no significant differences in growth performances among dietary treatments, the lowest whole-body Se level as well as whole-body Se-GPx activity was observed in fish fed diets without Se supplement, indicating a nutritional Se deficiency. These results, aside from growth outcomes, are in line with the findings of the current experiment. Increased dietary Se level led to the augmentation of both muscle Se level and plasma GPx activity. Also, a linearity between muscle Se concentration and dietary Se level did exist over the course of the feeding experiment. These findings may suggest that both muscle Se level and plasma GPx

activity can be used as a biological indicator in delineating Se status in marine carnivorous finfish species, particularly when fed PP-based diets. The implications of OS supplementation on growth indices of barramundi might be attributed to the antioxidant capacity of Se.

Haematological assays are useful diagnostic tools for examining physiological conditions of the fish (Silkin & Silkina, 2005). In the present experiment, no differences were observed for haematocrit, and the haematocrit values in the current experiment (32.40–34.73%) were within the normal range (30–45%), as suggested by Adams et al. (1993). Furthermore, leucocrit values may reflect the health condition of fish (Wedemeyer et al., 1983), thus malnutrition and increased susceptibility to disease are ascribed to low levels of leucocrit (Bandyopadhyay & Das Mohapatra, 2009 ; El-Asely et al., 2014). In this study, significant increases in leucocrit were observed in barramundi fed OS-supplemented diets, suggesting that OS may include particular compounds that stimulate leucocrit production. Leucocrit assessment is considered a rapid and inexpensive tool for evaluating fish health status (Adams et al., 1993).

Histologically, signs of Se deficiency include the proliferation of lipid deposition in the liver (Burk et al., 1995), as well as myopathy in skeletal muscle (Le & Fotedar, 2013b). It is thus interesting to note that the disruption in growth, tissue Se content and GPx activity corroborate the histological findings observed in the current experiment, where muscle damage and fatty liver were obvious in fish fed LM-based diets lacking OS supplementation. While fish fed LM_{25} diets exhibited moderate multifocal necrosis that was characterised by degenerating muscle bundles, those fed LM_{75} diets displayed even more severe necrosis of muscle fibres. Of additional note in this experiment is that at LM_{75} dietary group, where juvenile barramundi had the highest PP levels, there was the largest proportion of fish displaying skeletal disorder, and moreover their lesions were more intense. Previously, some authors have reported histological abnormalities in the muscle of marine finfish due to Se deficiency as a distortion of muscle fibres (Le & Fotedar, 2014b) and a variation in size of degenerating muscle fibres (Poston et al., 1976b). Rodger et al. (1991) suggested that accumulation of lactic acid in the muscle fibres were likely to appear when muscle fibres were damaged. This muscle-affecting acidosis might induce the

reduction in blood oxygen carrying capacity, as suggested by Root (1931). Sudden death syndrome (SDS) of Atlantic salmon (*S. salar*) was associated with mortality that could be the result of either malfunction of essential muscles or the anoxic condition from lactic acidosis, or an interaction of both (Rodger et al., 1991). Therefore, skeletal muscle integrity is used as an important indicator in fish health histological-based assessment.

Furthermore, GPx activity is also associated with the configuration and function of cell membranes (Wang et al., 2013). In the current study, the level of GPx activity in plasma significantly reduced with the decreasing level of OS supplementation in LM diets. The reduction in antioxidant capacity would be presumed to trigger a substantial implication on the tissue cellular structure of the studied fish. Thus, lipid droplet congregation in hepatocytes could be expected. Similarly, Wang et al. (2013) reported an accumulation of lipid peroxidation in the liver of fish fed Se-deficient diets. Atencio et al. (2009) demonstrated that the recovery of the cyanobacterial-induced histopathological changes primarily occurred with the maximum level of Se supplementation in most fish organs. Obviously, in the present study, the OS supplementation conferred protection against cellular damages when fish were fed with PP-based diets. Although needed in trace amounts, Se plays an important function as the constraint in the antioxidant enzyme biosynthesis. Under Se-deficient conditions, there would be a higher incidence of pro-inflammation that might expose fish to severe diseases.

6.5. Summary

On the basis of the results from the present study, it could be concluded that Se supplementation in comparatively high inclusion level of LM-based diets for barramundi enhance growth and health performances. The present findings also confirm that Se in FM-based diets is inadequate to achieve maximal growth and GPx antioxidant enzymatic capacity of fish. However, it is not clear whether Se supplementation is still needed if the LM is further processed into more refined products, for instance through fermentation process. Therefore, the effects of fermented LM with and without Se supplementation in the diets for barramundi should be further investigated.

CHAPTER 7: Growth, enzymatic glutathione peroxidase activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented soybean meal and organic selenium

This research is published in Fish Physiology and Biochemistry
Volume 43 (2017): 775-790

7.1. Introduction

Barramundi (*Lates calcarifer*) is one of the economically important marine finfish species in Asian and Australian aquaculture. Like the farming of other carnivorous species, the major source of dietary protein for this species comes from fishmeal (FM) due to its protein- and other nutrients-dense properties. However, FM has become the most costly protein ingredient in aquaculture feed (Sarker et al., 2013). Thus, as the aquaculture industry rapidly grows, further increases in both demand for and the price of FM are unavoidable (Tacon & Metian, 2015). The major concern is that this can lead to unsustainable fisheries (Olsen & Hasan, 2012). Therefore, plant protein (PP) ingredients have been widely used in aquaculture feed as FM substitutes. Among those, soybean meal (SBM) remains the most promising feedstuff to replace much of the FM protein in diets for aquaculture species.

SBM has been shown to be an excellent protein sources for herbivorous and omnivorous finfish (Elangovan & Shim, 2000 ; Fagbenro & Davies, 2001 ; Kim et al., 2007 ; Tomás-Vidal et al., 2011 ; Yuan et al., 2013); however, an imbalance in amino acids (AA) and the presence of antinutritional factors (ANF) such as trypsin inhibitor, lectins and phytate (Refstie & Storebakken, 2001) constrain the incorporation of levels of SBM as protein sources for finfish formulated diets, especially those diets intended for carnivorous finfish (Biswas et al., 2007 ; Boonyaratpalin et al., 1998 ; Bowyer et al., 2013a ; Bowyer et al., 2013b ; Collins et al., 2012 ; Hernández et al., 2007 ; Laporte & Trushenski, 2012 ; Nengas et al., 1996 ; Wang et al., 2006b). Soy-derived phytate (myo-inositol-hexaphosphate), for instance, has been demonstrated to reduce growth and feed utilisation in striped bass (*Morone saxatilis*), rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*) (Hossain & Jauncey, 1993 ; Papatryphon et al., 1999 ; Usmani & Jafri, 2002). Moreover, phytate's ability to bind to important minerals such as iron (Fe),

magnesium (Mg), manganese (Mn), zinc (Zn) and selenium (Se) (Fredlund et al., 2006) before they are absorbed in the gut prevents mineral bioavailability for fish growth (Anderson & Wolf, 1995 ; Kumar et al., 2012). Reduction in mineral absorption and growth has been evidenced in Atlantic salmon (*Salmo salar*) when fed with phytate-containing PP feedstuffs (Storebakken et al., 1998). In this regard, the effect of phytate-mineral interaction can be compensated by supplementation with dietary minerals (Satoh, 2007). Indeed, findings from our previous study have demonstrated that organic Se (OS) improved the utilisation of PP ingredients and allow comparatively higher levels of FM replacement in the diets of barramundi (*L. calcarifer*) (Ilham et al., 2016a).

Although only required in trace amounts, Se is a fundamental nutrient for normal growth, physiological function, and cellular metabolism in fish (Gatlin et al., 1986 ; Watanabe et al., 1997). Se serves as an important component for the regulation of the antioxidant enzyme glutathione peroxidase (GPx), which protects membranes at both the cellular and subcellular level from oxidative damage (Jaramillo Jr et al., 2009). Fish may absorb minerals directly from the surrounding water, but diet is the primary source of Se (Lall, 2003). PP-based diets lacking dietary Se supplementation resulted in depressed growth and reduced GPx activity in hybrid striped bass (*M. chrysops* × *M. saxatilis*) (Cotter, 2006), most probably due to the chelating action of phytate. However, phytate not only chelates essential minerals, but also interferes with the absorption and digestibility of protein and AA (Selle et al., 2012), which may affect protein structure, enzymatic activity and proteolytic digestibility. Therefore, degradation of phytate in SBM would eliminate the antagonistic effect of phytate-mineral and phytate-protein complex interaction and improve the nutritive value of the SBM.

One approach to avoiding the detrimental effect of phytate-containing SBM might be its removal from the meal through fermentation process (Kumar et al., 2012) which allows the action of microorganisms to hydrolyse phytate to lower inositol phosphates (Hotz & Gibson, 2007). Inoculation of corn-SBM with brewer's yeast induced a massive production of phytase, thus degrading phytate phosphorus (P) in diets for pigs (Chu et al., 2009). Microbial fermentation of PP feedstuffs has been applied to improve organoleptic quality, mineral bioavailability and protein quality

and digestibility (García-Mantrana et al., 2015) to finfish species. Lactic acid fermentation of corn flour promoted removal or inactivation of ANF (Sokrab et al., 2014). Lactic acid bacteria also produce numerous low-molecular-weight compounds that potentially enhance mineral absorption through the formation of soluble ligands while concomitantly generating a low pH that elevates the activity of endogenous phytase from PP meal (Hotz & Gibson, 2007). Better growth performance and feed utilisation were obtained when fermented SBM was fed to Japanese yellowtail (*Seriola quinqueradiata*) in comparison to non-fermented diets (Shimeno et al., 1993).Yamamoto et al. (2010) observed that microbial fermentation of SBM provided protection against SBM-induced atrophy and histological abnormalities of rainbow trout (*Oncorhynchus mykiss*). Further, in a Nile tilapia (*Oreochromis niloticus*) study, *Saccharomyces cerevisiae*-bioprocessed SBM yielded higher growth performance and better FCR (Hassaan et al., 2015). Sharawy et al. (2016) also reported that 50% FM protein can be replaced with *S. cerevisiae*-fermented SBM in the diets of Indian white shrimp (*Fenneropenaeus indicus*) without hampering their growth performance. *S. cerevisiae* is the most important microorganism in the production of fermented food (Lund & Baird-Parker, 2000 ; Schuller, 2010) due to its high tolerance of alcohol and of hot and anoxic environments (Goddard & Greig, 2015). It is apparent that fermentation not only enhances biological activity but also diminishes ANF in raw ingredients.

Fermentation has been practiced to improve the quality of PP-based aquaculture feed. However, there are no published data available on the effect of fermented SBM simultaneously supplemented with OS in barramundi. Therefore, the present study was conducted to evaluate the efficacy of fermented SBM-derived protein sources and OS supplementation as a means of nutritive value enhancement. The effects on growth, enzymatic GPx activity and blood physiology were investigated.

7.2. Material and methods

The methodology of this experiment was approved by the Animal Ethics Committee of Curtin University (Approval Number: AEC-2013-07). The experiment was conducted at the Curtin Aquatic Research Laboratory (CARL), Curtin University, WA, Australia.

7.2.1 Preparation of fermented SBM

SBM ingredient was supplied by Specialty Feeds (Glen Forrest, WA, Australia). SBM were powdered and sieved through a 0.5-mm sieve and used as the raw material for fermentation. SBM fermentation were performed by a modification technique of Hassaan et al. (2015), in three independent replicates. In each replicate, 2 kg SBM, 66 mg of baker's yeast, *S. cerevisiae*, with a cell density of 3×10^6 colony forming units (CFU) g^{-1} meal, and 1.6 L of distilled water were homogenized in a food mixer for 15 min. The mixture was placed in a rectangular glass container covered with aluminium foil and incubated at 30ºC for 5 days. Then the product of fermentation was dried at 60ºC for 24 h and used as an ingredient for feed preparation. A representative sample was taken for AA and ANF analysis of the ingredients as shown in Table 7.1.

Table 7.1. AA (g 100 g^{-1} protein) and proximate composition (%) of FM, SBM and fermented SBM

	FM	SBM	Fermented SBM
Arginine	4.35	3.69	3.94
Histidine	2.09	1.23	1.54
Isoleucine	3.04	2.25	2.65
Leucine	5.17	3.88	4.65
Lysine	4.77	2.83	2.92
Methionine	1.86	0.58	0.79
Phenylalanine	2.87	2.61	3.01
Threonine	3.19	1.89	2.38
Valine	3.26	2.14	2.47
Alanine	4.42	2.04	2.48
Aspartic acid	6.20	5.48	6.35
Glutamic acid	8.25	8.96	10.33
Glycine	4.73	1.94	2.34
Proline	3.81	3.01	3.94
Serine	3.05	2.64	3.08
Phytic acid (%)	–	1.18	0.37

7.2.2 Experimental diet

Four isonitrogenous and isocaloric diets were formulated to contain 49% crude protein and 20 MJ kg^{-1} gross energy. SBM was used to replace 75% FM protein fermented or non-fermented (SBM and FSBM, respectively) and supplemented with organic Se (OS) (SBM_{OS} and $FSBM_{OS}$, respectively). Supplementation level of 2 and 2.5 g OS kg^{-1} was based on the findings of our previous study to maintain the required Se level for maximum growth (Ilham et al., 2016a). A FM-based diet formulated for barramundi according to known nutritional requirements (Catacutan & Coloso, 1995 ; Catacutan & Coloso, 1997 ; Glencross, 2006) was used as the reference diet, without SBM or OS supplementation.

Experimental diets were prepared according to standard CARL methods. All dry ingredients were finely ground through a 0.5 mm mesh screen and mixed manually before transferred to a food mixer for 15 min. 5 g kg^{-1} chromic oxide (Cr_2O_3, Thermo Fisher Scientific, Scoresby, Vic., Australia), which was used as an inert indicator for digestibility measurement, was dissolved in 100 mL of distilled water and sprayed on the mash during mixing. Then, mixed ingredients were pelleted using a laboratory pelleting machine to the desired size, air-dried, and stored at 4°C until use. 50 g of each diet was sampled for proximate composition and Se analysis of the diets following the methods of AOAC (1990) as shown in Table 7.2.

7.2.3 Fish and experimental condition

Juvenile barramundi (average individual weight of 5.04 g) were supplied by the Australian Centre for Applied Aquaculture Research (ACAAR), Fremantle, Western Australia (WA), Australia. Fish were acclimated to feeding and rearing conditions for seven days before the commencement of the trial. During the acclimation period, all fish were fed the commercial diet twice daily. Following acclimation, fish were starved for 24 h, bulk-weighed, and a total of 300 healthy juvenile barramundi were randomly stocked into fifteen 300-L experimental tanks (20 fish $tank^{-1}$). The tanks were supplied with recirculated water from an external biofilter (Fluval 406, Hagen, Italy) at a rate of 10 L min^{-1}. All experimental tanks were equipped with constant aeration and pure oxygen (compressed oxygen, BOC, Perth, WA). Water quality parameters such as temperature, dissolved oxygen, and salinity were measured daily and maintained at 28-29 ºC, >5 mg L^{-1}, 32-34 ppt, respectively. The photoperiod was

set at 12 hours of fluorescent light per day. During the feeding trial, triplicate groups were hand-fed the respective experimental diets and the control diet *ad libitum* during two feeding sessions a day at 0900h and 1500 h, 7 days a week for 75 days. Fish were bulk-weighed every 15 days to record growth. Dead fish were weighed and recorded in order to adjust the calculation of the feed conversion ratio (FCR) and survival.

7.2.4 Digestibility assessment

To analyse the effect of dietary treatment on digestibility, one week before the end of the feeding trial faecal matter was collected immediately prior to the morning feeding using stripping techniques (Austreng, 1978). Faecal collections from individuals were pooled by tank and quickly stored at −20ºC. Prior to analysis, the faecal samples were dried to constant weight at 105 ºC. Apparent digestibility coefficients (ADCs) were measured using the indirect method (Cr_2O_3) as suggested by Cho et al. (1982).

Table 7.2. Feed ingredients and chemical composition of the experimental diets

Ingredient[a] (g kg^{-1} DM basis)	Diets[b]				
	SBM	SBM_{OS}	FSBM	$FSBM_{OS}$	FM
FM	180	180	200	200	460
SBM[c]	430	430	395	395	–
Wheat gluten	100	100	100	100	100
Wheat flour	10	10	10	10	80
Casein	120	120	120	120	120
Fish oil	110	110	110	110	100
Wheat starch	25	23	25	25	65
Cellulose	–	–	15	12.5	50
Se-free premix[d]	20	20	20	20	20
Sel-Plex[e]	–	2	–	2.5	–
Chromic oxide	5	5	5	5	5
Proximate composition (%)					
Dry matter	90.38	89.72	88.08	88.10	85.17
Ash	7.43	7.22	6.37	6.38	8.25
Crude protein	49.33	49.31	50.13	50.15	49.19
Crude lipid	14.52	14.55	14.75	14.73	15.06
Gross energy	20.13	20.07	20.54	20.55	21.33
Se (mg kg^{-1})	1.55	3.51	1.03	3.52	3.37

[a] Supplied by Specialty Feeds, Perth, WA, except for Sel-Plex® and chromic oxide, obtained from AllTech, Lexington, Kentucky, USA and Thermo Fisher Scientific, Scoresby, Vic., Australia, respectively.

[b] SBM (soybean meal); SBM_{OS} (SBM+OS); FSBM (fermented SBM); $FSBM_{OS}$ (fermented SBM+OS); FM (fishmeal).

[c] Solvent-extracted; Malaysian origin

[d] Contains the following (as g kg^{-1} of premix): calcium, 5.5; phosphorus, 17.5; iron, 10; magnesium, 2.8; copper, 1.5; iodine, 0.15; manganese, 9.5; zinc, 25; cobalt, 0.13; vitamin A retinol, 100 IU; vitamin D3, 100 IU; vitamin E, 6.25; vitamin K, 1.6; vitamin B1, 1; vitamin B2, 2.5; niacin, 20; vitamin B6, 1.5; calcium, 5.5; biotin, 0.1; folic acid, 0.4; inositol, 60; vitamin B12, 0.002; choline, 150; ethoxyquin, 0.125.

[e] 1 kg Sel-Plex® contains 1 g of OS.

7.2.5 Sampling and chemical analysis

At the end of the trial, all fish were deprived of food for 24 hours to achieve a basic metabolite state. The fish were then anaesthetised with tricaine methanesulfonate (MS-222, Sigma-Aldrich, Castle Hill, NSW, Australia) at 100 mg L^{-1} followed by individual weighing and counting to compute the weight gain (WG), specific growth

rate, feed conversion ratio (FCR) and survival. Blood samples from three fish in each tank (9 fish per dietary treatment) were then withdrawn by caudal vein puncture with a 1 mL plastic syringe. The extracted blood was transferred to BD Vacutainer® blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for haematology. Subsamples of whole blood were immediately analysed for haemoglobin (Hb), haematocrit and leucocrit. Remaining blood samples were then centrifuged for 5 min at 12,000 rpm at 4 ºC, and the plasma samples collected were stored at 4 ºC until subsequent blood chemistry analyses were performed. Erythrocyte (red blood cell) glutathione peroxidase (GPx) activity was quantitatively assayed using the Randox Laboratories test combination (Ransel, Antrim, United Kingdom).

Blood samples were sent to the Animal Health Laboratories (South Perth, Western Australia) for plasma enzyme and chemistry analysis. The assays were run on an Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Mt Waverley, VIC, Australia). Each of the assays used was a standard kit developed for the auto-analyser. The tests performed included creatinine kinase (CK) (Olympus kit Cat. No. OSR6179), alanine aminotransferase (ALT) (Olympus kit Cat. No. OSR6107), urea (Olympus kit Cat. No.OSR6134), creatinine (Olympus kit Cat. No. OSR6178), phosphate (Olympus kit Cat. No. OSR6122) total protein (Olympus kit Cat. No. OSR6132), and albumin (Olympus kit Cat. No. OSR6102).

The proximate compositions of the diet and faecal samples were determined based on Association of Official Analytical Chemists procedures (AOAC, 1990). Dry matter was determined by oven drying to constant weight at 105ºC; crude ash by combustion at 550 ºC; crude protein content (N × 6.25) by the Kjeldahl digestion method; crude lipid content by the Soxhlet technique; and gross energy content by an IKA oxygen bomb calorimeter (Heitersheim, Germany). AA content of the diets was determined after samples were hydrolysed in HCl (Barkholt & Jensen, 1989 ; Rayner, 1985). Analyses were performed on an Agilent 1100 series high-performance liquid chromatography (HPLC, Agilent Technologies, Germany) system using conditions similar to those described by Gratzfeld-Huesgen (1998). Se was analysed at the Intertek Genalysis Laboratory (Perth, Australia) using inductively coupled plasma atomic emission spectrometry (ICP-MS). Phytate content

of the samples was determined using automated High-performance liquid chromatography (HPLC) analysis as suggested by Kwanyuen and Burton (2005).

7.2.6 Calculations

Growth and feeding performances were measured using the calculated parameters as follows. Specific growth rate = 100 × [ln (final weight) − ln (initial weight)/days]. Weight gain (WG) = 100 × [(final weight − initial weight)/initial weight]. Feed intake (FI) = [(dry diet given − dry remaining diet recovered)/number of fish]/days. Feed conversion ratio (FCR) = feed intake/weight gain. Survival = 100 × (final number of fish/initial number of fish).

7.2.7 Statistical analysis

All data concerning the effects of SBM products, Se level and their interactions on growth, feeding, digestibility, haematological and physiological responses were subjected to two-way analysis of variance (ANOVA). Assumptions of homogeneity of variances were checked using Levene's equal variance test. One-way ANOVA was performed to compare the control FM diet against each experimental diet. When significant effects were obtained for a factor, the Duncan test was used to compare the reference diet against each test diet. Means were considered to be significantly different when $P < 0.05$. Percentage data were computed using arcsine transformations. All statistical analyses were carried out using SPSS (version 22, IBM Inc., Australia).

7.3. Results

7.3.1 Growth performances

Data for FW, SGR, WG, FI, FCR and survival are presented in Table 7.3. At the end of the rearing trial, the survival of juvenile barramundi in all dietary treatments was at least 95%. An interaction between the SBM type and OS level ($P < 0.05$) was observed in this study. In fish fed diets supplemented with OS (SBM_{OS} and $FSBM_{OS}$), FW, SGR and WG were higher in fish fed the fermented SBM ($FSBM_{OS}$) than in those fed the non-fermented SBM (SBM_{OS}). The FW, SGR and WG of fish fed the SBM_{OS} and $FSBM_{OS}$ diets were similar to those of fish fed the FM diet ($P > 0.05$). Without OS supplementation, the FW, SGR and WG decreased ($P < 0.05$);

however, when OS was added to the diet ($FSBM_{OS}$), the SGR and WG increased to levels similar to those of the FM-based diet. Although all fish appeared to maintain equivalent FI throughout the experimental period, supplementation of OS to the fermented SBM diet was also able to improve the FCR similar to that of the reference diet.

7.3.2 Digestibility

In this study, the ADC of protein was higher in the fish fed the fermented SBM diets (FSBM and $FSBM_{OS}$) than in those fed the non-fermented SBM diets (SBM and SBM_{OS}) ($P < 0.05$). The ADC of fish fed the FSBM and $FSBM_{OS}$ diets was not different from that of fish fed the reference diet. Likewise, the ADC of DM was higher in the FM and fermented SBM groups than in the non-fermented SBM groups. In addition, the ADC of protein of the fish fed the non-fermented SBM was lower than that of those fed the FM diets ($P < 0.05$). However, the ADC of DM and lipids were not significantly different among the tested diets and when compared with the reference diet (Table 7.4).

7.3.3 Muscle Se content

At the end of the feeding experiment, muscle Se content was significantly affected by OS supplementation ($P < 0.05$). Higher muscle Se content was found in the fish fed SBM_{OS} and $FSBM_{OS}$ diets, being 0.52 mg kg^{-1} and 0.51 mg kg^{-1}, respectively. In addition, muscle Se content of the fish fed the OS-supplemented diets was higher than that of those fed the reference diet (Fig. 7.1).

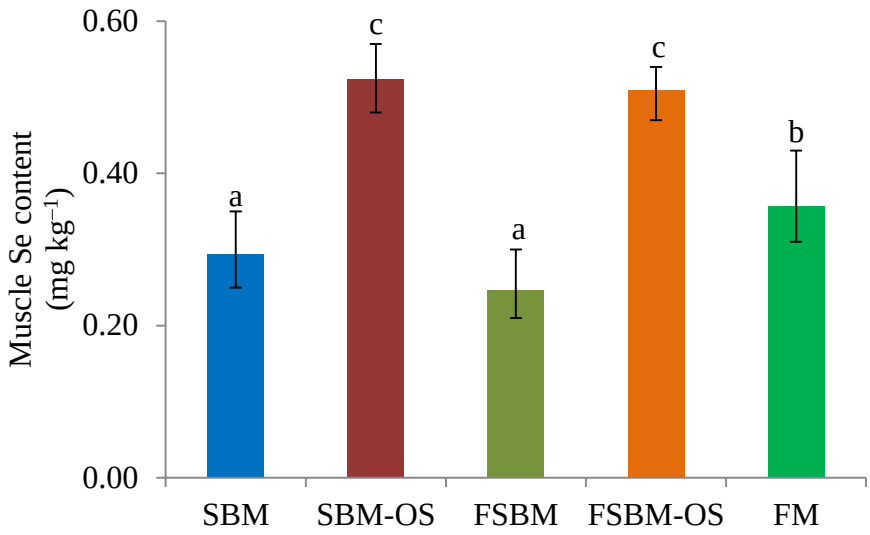

Figure 7.1. Muscle Se content of fish fed the experimental and FM reference diets. Different letters above the bars denote significant differences between diet groups at the $P < 0.05$ level.

Table 7.3. Growth performance, survival and feed utilisation of juvenile barramundi fed test diets formulated with soybean meal (SBM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	SBM	SBM_{OS}	FSBM	$FSBM_{OS}$	SEM	SBM	OS	SBM×OS	
FW	36.29*	38.2	36.41*	41.27	0.495	0.014	0.000	0.016	40.10
SGR	2.62*	2.69*	2.60*	2.81	0.035	ns	0.005	0.048	2.80
WG	621.41*	650.26	597.29*	725.91	18.964	ns	0.004	0.040	720.68
FI	0.61	0.62	0.61	0.61	0.011	ns	ns	ns	0.62
FCR	1.47**	1.36	1.46**	1.26	0.031	ns	0.033	ns	1.25
Survival	97	95	95	98	1.559	ns	ns	ns	100

Two-way ANOVA performed involving only the experimental diets.

ns = not significant; SEM = standard error of the mean.

Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$).

FW (final weight, g); SGR (specific growth rate, % body weight day^{-1}); FI (feed intake, g $fish^{-1}$ day^{-1}); WG (weight gain, %); FCR (feed conversion ratio); survival (%).

Table 7.4. Apparent digestibility coefficients (ADC) of dry matter, protein and lipid of juvenile barramundi fed test diets formulated with soybean meal (SBM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	SBM	SBM_{OS}	FSBM	$FSBM_{OS}$	SEM	SBM	OS	SBM×OS	
DM	85*	86	89	88	0.601	ns	ns	ns	90
Protein	90*	91*	94	94	0.570	0.010	ns	ns	95
Lipid	95	95	94	95	0.180	ns	ns	ns	96

Two-way ANOVA performed involving only the experimental diets.

ns = not significant; SEM = standard error of the mean.

Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$).

DM (dry matter, %); protein (%); lipid (%).

7.3.4 GPx activity and haematology

Table 7.5 displays the GPx activity and haematological indices of the fish following the course of the feeding trial. GPx activity was significantly influenced by the OS level in the experimental diets; GPx activity was greater in the fish fed diets supplemented with OS. However, the GPx activity of the fish fed the FM diets was higher than that of those fed diets unsupplemented with OS (SBM and FSBM), but lower than that of those fed OS-supplemented diets (SBM_{OS} and $FSBM_{OS}$). Furthermore, haematocrit was significantly affected by the OS supplementation and the haematocrit of fish fed the $FSBM_{OS}$ diets was lower than that of fish fed the FM diets ($P < 0.05$). Hb and leucocrit were not significantly affected by SBM type and OS supplementation or by their interaction ($P > 0.05$).

7.3.5 Blood biochemistry status

As shown in Table 7.6, except for ALT, all other blood biochemistry parameters measured in the present study were not affected by either the SBM type (fermented or non-fermented) or by dietary OS supplementation ($P > 0.05$). The activity of ALT was affected by OS supplementation; fish fed OS-supplemented diets attained lower ALT levels. In contrast, ALT activities of fish fed diets lacking OS supplementation were higher than those fed the other diets, including the FM reference diet. Furthermore, the CK of all experimental diets was significantly higher than that of those fed the FM reference diet ($P < 0.05$). Compared with the fish fed the OS-supplemented diets, the TP decreased in the fish fed the SBM diet without fermentation or OS supplementation.

Table 7.5. GPx activity and haematological indicator of juvenile barramundi fed test diets formulated with soybean meal (SBM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	SBM	SBM_{OS}	FSBM	$FSBM_{OS}$	SEM	SBM	OS	SBM×OS	
GPx	171.3*	187**	175*	185.7**	2.556	ns	0.008	ns	180
Hb	72	73	70	71	0.534	ns	ns	ns	71
Haematocrit	31.3	34.67**	31.08	35.76**	0.502	ns	0.000	ns	30.85
Leucocrit	1.45	1.31	1.32	1.57**	0.082	ns	ns	ns	1.33

Two-way ANOVA performed involving only the experimental diets.

ns = not significant; SEM = standard error of the mean.

Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$).

Hb (haemoglobin, g dL^{-1}); haematocrit (%); leucocrit (%), GPx (U g^{-1} Hb).

Table 7.6. Blood chemistry of juvenile barramundi fed test diets formulated with soybean meal (SBM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	SBM	SBM_{OS}	FSBM	$FSBM_{OS}$	SEM	SBM	OS	SBM×OS	
CK	3207**	2944**	3025**	2881	137.13	ns	ns	ns	2676
ALT	15**	12	16**	12	1.067	ns	0.047	ns	13
Urea	8	10	9	8	1.027	ns	ns	ns	9
Creatinine	21	20	18	22	0.755	ns	ns	ns	21
Phosphate	4	4	3	4	0.246	ns	ns	ns	4
TP	41	45**	43**	44**	0.865	ns	ns	ns	41
Albumin	15	16	15	17**	1.518	ns	ns	ns	15
A/G ratio	0.7	0.6	0.6	0.7	0.078	ns	ns	ns	0.6

Two-way ANOVA performed involving only the experimental diets.

ns = not significant; SEM = standard error of the mean.

Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$).

CK (creatinine kinase, U L^{-1}); ALT (alanine aminotransferase, U L^{-1}); urea (mmol L^{-1}); creatinine (μmol L^{-1}); phosphate (mmol L^{-1}); TP (total protein, g L^{-1}); albumin (g L^{-1}); A/G (albumin to globulin).

7.4. Discussion

SBM is the most important protein source to feed farmed animals in the world today (Tacon & Metian, 2015). PP ingredients such as SBM contain considerable amounts of antinutritional compounds and toxic components, such as phytates, tannins, saponins, lectins and trypsin inhibitors (Francis et al., 2001). Phytate interferes with Ca, Fe, Mg, Zn and Se absorption due to its ability to chelate divalent cationic minerals (Connelly, 2011). However, phytate can be degraded through fermentation of plant-derived protein ingredients (Reddy & Pierson, 1994). As shown in Table 1, the phytate content of the SBM was reduced by 68% after fermentation. Similarly, the findings of previous studies also suggested that fermentation is an effective technique for significantly eliminating the effect of antinutritional phytates in PP ingredients (Sharawy et al., 2016 ; Sun et al., 2015b ; Van Vo et al., 2015). A combined effect of both endogenous and microbial phytases allows the removal of phytate during the fermentation process (Raes et al., 2014). Thus, dietary FSBM had been successfully applied for terrestrial livestock (Feng et al., 2007 ; Jeong et al., 2015) and aquatic animals (Azarm & Lee, 2014 ; Yuan et al., 2013 ; Zhang et al., 2014b).

Substantial elevation in the level of amino acids (AA) can be attributed to the hydrolysis process (Rayner, 1985), which would have enhanced the nutritional quality of SBM, leading to the enrichment of protein content of the fermentation product. Moreover, increased production of the cell mass of yeast can boost the generation of protein within the yeast population. A similar improvement of protein was found in yeast fermented SBM by Hassaan et al. (2015). Dietary protein has been considered as the most fundamental nutrient for the growth of fish and crustaceans (Bowyer et al., 2013c ; Hardy, 2010 ; NRC, 2011). The quality of SBM protein in terms of AA content was noted to be identical to that of barramundi muscle (Williams et al., 2003a). Fermentation is important for attaining high nutritional value and to modify the functional properties of SBM protein.

In the present study, the growth of barramundi fed the fermented SBM-based diet supplemented with OS ($FSBM_{OS}$) significantly improved compared to fish fed other diets (SBM, SBM_{OS} and FSBM) and was similar to those fed the FM reference diet.

Interestingly, the reduced growth in fish fed the FSBM diet was unexpected, which might be attributed to the effect of fermentation on mineral bioavailability, as observed for Se. Phytate, the major storage form of phosphorus (P) in plant-derived tissues, may reduce the availability of dietary essential minerals, including Se (Kumar et al., 2012). An incomplete removal of phytate during fermentation might have increased the mineral binding capacity of phytate, and consequently led to the depletion of bioavailability of minerals required for growth. These findings strongly indicate that supplementation of OS is necessary not only for solvent-extracted SBM, as observed in our previous study (Ilham et al., 2016c), but even for the more refined products, such as fermented SBM, as found in the present study. Indeed, the muscle tissue Se contents of fish fed the $FSBM_{OS}$ diet were higher than that of fish fed the other diets, suggesting that trace mineral (Se) utilisation was enhanced by the OS supplementation.

Moreover, OS supplementation may promote high absorption of AA-chelated Se in the mucosal tissues, leading to the increased production of hydrolytic enzymes, such as gastro-intestinal (GI) glutathione peroxidase activity (GPx), which induces the enhancement of feed digestion. Thus, it is not surprising that OS supplementation correspondingly affected FCR, as observed in the present study. The improved FCR in fish fed SBM_{OS} and $FSBM_{OS}$, combined with the improved WG in fish fed these two diets, suggest that SBM-based diets supplemented with OS can replace high amounts of FM without a deleterious impact. To our knowledge, this is the first study to have examined the influence of OS as feed supplement in SBM-based diets on the growth and blood physiology of marine carnivorous finfish species. Se deficiency in PP sources can be a limiting factor in the utilisation of SBM protein in finfish diets (Fontagne-Dicharry et al., 2015). SBM or casein-based diets lacking Se supplementation has been reported to decrease growth and GPx activity of hybrid striped bass (*Morone chrysopx* × *M. saxatilis*) (Cotter et al., 2008). Our previous study suggested that when 75% lupin meal was incorporated to replace FM protein in the diets, the Se level that was needed to maintain maximum growth in juvenile barramundi was 3.5–4.5 mg kg^{-1} diet (Ilham et al., 2016a). The Se level of FSBM diet (1.03 mg Se kg^{-1}) was below the above proposed level for PP-based diets, and thus did not satisfy the requirements of barramundi.

In most aquatic animals, the dietary Se requirement is dependent upon species, age, the form of Se ingested, vitamin E content of the diet, and pathway and duration of exposure to Se (NRC, 2011). The recommended dietary Se level has been established for several finfish species, including Atlantic salmon (*S. salar*) (<2.1 mg kg^{-1}) (Lorentzen et al., 1994), cobia (*Rachycentron canadum*) (0.8 mg kg^{-1}) (Liu et al., 2010), cutthroat trout (*Oncorhynchus clarkii*) (9.2 mg kg^{-1}) (Hardy et al., 2010), grouper (*Epinephelus malabaricus*) (0.7 mg kg^{-1}) (Lin & Shiau, 2005), hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (1.2 mg kg^{-1}) (Jaramillo Jr et al., 2009), largemouth bass (*Micropterus salmoide*) (1.60–1.85 mg kg^{-1}) and yellowtail kingfish (*Seriola lalandi*) (5.56 mg kg^{-1}) (Le & Fotedar, 2013b). Furthermore, it is commonly accepted that OS (i.e. selenomethionine, Se-Met) is more bioavailable than inorganic Se to fish (Le & Fotedar, 2014a ; Wang & Lovell, 1997). The OS utilised in the present study was in the form of selenised yeast (Sel-Plex®, Alltech Inc., Lexington, Kentucky, USA) that contains a mixture of selenoamino acids with Se-Met representing more than 50% of the total Se (Lyons et al., 2007). Trace element amino acid chelates (AAC) have been widely applied in animal nutrition. One desirable feature of the use of the AAC is that they can enhance mineral bioavailability and promote growth and tissue mineral deposition in many land-based and aquatic animals (Sarker et al., 2007 ; Satoh, 2007).

Irrespective of OS supplementation, the fermentation method can improve crude protein digestibility of SBM for barramundi. The ADC of protein was observed to be comparable to the reference diet for fermented SBM diets (FSBM and $FSBM_{OS}$), whereas in the non-fermented diets (SBM and SBM_{OS}), the ADC of protein was lower in comparison to those of the reference diet. Zhuo et al. (2014) suggested that *Lactobacillus* spp. fermented SBM in groupers (*Epinephelus coioides*) could improve the ADC of protein and DM. Yuan et al. (2013) reported that better ADC of protein of SBM for Chinese sucker (*Myxocyprinus asiaticus*) diets can be obtained through the fermentation process. Brown et al. (1986) reported that PP ingredients were more digestible than those of animal sources, whereas Hernández et al. (2007) demonstrated that the opposite was true. However, similar ADC of protein was found between non-fermented SBM and fermented SBM in tilapia (Zhou & Yue, 2012). In addition, Yuan et al. (2010) noted that the ADC of protein for fermented SBM was similar to that for FM, but superior to that for traditional SBM in diets for the

Chinese sucker (*M. asiaticus*). These discrepancies are probably attributable to differences in microbial strains, variation in species, fermentation conditions and drying techniques. Therefore, the ADC values reported in this study may suggest that fermented SBM has the potential to become the dominant protein source in cultured finfish feeds. Lower ADC of protein in the non-fermented diets, as observed in the present experiment, might have been induced by the presence of the antinutritional phytate in SBM, which can hamper the digestibility of nutrients. Diets that are defectively digested would be of inadequate nutritional value to the fish. In line with the results of the present study, further study should examine the effect of Se supplementation in PP-based diets on digestive enzymes such as pepsin and trypsin.

The use of fermented PP products has been reported to promote the beneficial effects of non-specific immune responses and antioxidant activities of fish (Ashida & Okimasu, 2005 ; Ashida et al., 2006 ; Kim et al., 2010). Increased antioxidant capacity was found in red sea bream (*Pagrus major*) fed diets containing fermented SBM, although the mechanism of this circumstance remained unclear (Abdul Kader et al., 2011). This result is in accordance with a study by Azarm and Lee (2014), which suggested that fermented SBM triggered increased enzymatic antioxidant of GPx via access to isoflavons by microbial activity for juvenile black sea bream (*Acanthopagrus schlegeli*). However, in the present study, the enhancement of enzymatic antioxidant activity of GPx was modified by OS supplementation rather than by the fermentation process. The relationship between dietary OS and GPx activity was demonstrated. The enzymatic activity level of GPx of fish fed diets supplemented with OS was superior to the other tested diets and to the reference diet. These findings are in line with the antioxidant GPx responses to dietary Se as reported for many fish species (Bell et al., 1987 ; Elia et al., 2011 ; Han et al., 2011 ; Hao et al., 2014 ; Le & Fotedar, 2014b ; Ribeiro et al., 2012 ; Wang et al., 2007 ; Zhou et al., 2009). GPx has strong radical-scavenging activity against hydrogen peroxides, organic hydroperoxides or lipid peroxides (Takahashi & Cohen, 1986). In addition, dietary Se intake modifies the mRNA expression and GPx activity, and Se insufficiency immediately reduces all cellular GPx activity in cod (*Gadus morhua*) (Penglase et al., 2010). Because Se in the form of selenocysteine exists in the active site of this enzyme (Rotruck et al., 1973), it is conceivable that Se deficiency leads to a reduction in enzymatic antioxidant capacity. Antioxidant GPx activity in plasmid

tissues is linearly related to dietary Se intake (Gatlin & Wilson, 1984 ; Hao et al., 2014), unless at an extreme level. Further, muscle Se level has also been linked to GPx activity in several fish species, since muscle tissue is a strong Se accumulator (Hilton et al., 1980 ; Kucukbay et al., 2009 ; Zhu et al., 2012). Thus increased muscle Se level and GPx activity with elevated dietary Se levels were found in this study, suggesting that both muscle Se level and plasma GPx activity can be used as biological indicators for describing the bioavailability of Se in carnivorous finfish species. The effects of OS supplementation on the growth performances of juvenile barramundi could be related to the antioxidant role of Se.

The effect of OS supplementation in PP-based diets on the haematological indices, particularly of marine carnivorous finfish species, has rarely been researched. Haematological characteristics are important tools that can be used to detect pathophysiological states in fish (López et al., 2015). Thus, changes in haematology of cultured fish in response to diets provide useful information in monitoring fish health, which is a key issue in aquaculture.

However, significant differences in the values of haematocrit and leucocrit were observed in the present study. Haematocrit values were increased in fish fed diets supplemented with OS. These results may be related to the elevated TP in fish fed the SBM-based diets, as had also been found when totoaba juveniles (*Totoaba macdonaldi*) were fed with soy protein concentrate (SPC) diets (López et al., 2015). Blaxhall (1972) proposed the option of using haematocrit status as an effective and sensitive index in aquaculture for testing for an anaemic condition. The high level of haematocrit in fish fed OS-supplemented diets signifies a high oxygen capacity of blood as compared with those fed diets lacking OS supplementation. Interestingly, the haematocrit values of fish fed the FM reference diet were similar to those fed OS-deficient diets. These findings may suggest that although Se content from FM was relatively high, the Se could not be utilised to enhance haematocrit levels in fish fed the FM-based diets. Webster and Lim (2002) described that FM-derived Se had lower bioavailability due to Se binding with heavy metals, which might have been the case in this study. Haematocrit values in the present study were within the range of 30.85% to 35.76%; however, the normal range of haematocrit values for fish are usually between 30% and 45% (Adams et al., 1993). Further, considerable rises in

leucocrit were noted in fish fed OS-supplemented diets, indicating that OS may contain certain compounds that stimulate leucocrit production. Leucocrit assays have been used to evaluate the health status of Nile tilapia (*Oreochromis niloticus*) (El-Asely et al., 2014).

In fish, variations of some plasma enzymes can be used as a diagnostic tool for investigating dysfunction in organs. CK plays an important role in the energy homeostasis of cells (Shahsavani et al., 2008) and has been used as a biochemical indicator of muscle damage in tilapia (Chen et al., 2003). In the present study, with the exception of the $FSBM_{OS}$ diet, the SBM-based diets caused substantial elevations in plasma CK activity relative to the FM reference diet. These results suggest that the inclusion of SBM in the diet might have induced muscle-related injuries. It is well accepted that due to phytate's complexing action with minerals, PP ingredients such as SBM are typically deficient in Se, which may lead to Se-induced muscle damage or myopathy. As noticed in this study, supplementation of OS appeared to maintain a CK activity level similar to that of the FM reference diet. Thus the association between CK activity and Se status in marine carnivorous finfish could be established with respect to muscle health. In line with this, we have observed in our previous study that the potential occurrence of muscle necrosis was high when juvenile barramundi were fed with high lupin meal diets lacking Se supplementation (Ilham et al., 2016a).

The elevation of ALT activity in response to exogenous agents seems to illustrate liver dysfunction (Shi et al., 2006). In this study, plasma ALT activity was significantly lower in fish fed SBM_{OS} and $FSBM_{OS}$ diets than in OS-deficient groups. To our knowledge, there has been no study to relate the ALT activity and Se levels in carnivorous marine finfish. However, Kenari et al. (2013) reported that ALT activity was significantly decreased in Caspian brown trout (*Salmo trutta caspius*) fed diets supplemented with nucleotides. Similarly, decline in ALT activity was documented in a study of barramundi fed nucleotide-supplemented diets by Glencross and Rutherford (2010). The reduction in plasma ALT activity in the present study might reveal that the dietary OS promotes the potential to benefit the health of fish by boosting liver function.

Plasma proteins such as albumin and globulin serve as the most important fraction in the transportation of substances and in the maintenance of a vigorous immune system (Andreeva, 2012). In general, an increase in the plasma TP levels is believed to be a biomarker of an improved innate immunity in fish (Soltanzadeh et al., 2015). In addition, TP is an important indicator of the metabolism of diet, and its decline may be caused by poor nutrient digestion and metabolism. In this study, SBM_{OS}, FSBM and $FSBM_{OS}$ diets increased the TP of fish compared to the FM reference diet over the course of the feeding experiment. In the same way, OS supplementation in SBM resulted in increased albumin concentration; however, this was not the case for the non-fermented SBM diet. Although the TP levels obtained in this study were somewhat similar to the TP levels of barramundi fed diets containing lupin kernel meal, as reported by Glencross et al. (2011), it is difficult to demonstrate whether variations in TP and albumin were mediated by the fermentation of SBM or by the supplementation of OS. Further research is required to elucidate those mechanisms.

7.5. Summary

The present study demonstrates that high level of fermented SBM combined with supplementation of OS can improve the growth performance of barramundi and play an essential role in adjusting enzymatic antioxidant GPx activity and blood haematological and biochemistry status. Dietary supplementation with OS in other PP products may allow a considerable replacement level of FM. This deserves to be studied further.

CHAPTER 8: Growth, enzymatic glutathione peroxidase (GPx) activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented lupin meal supplemented with organic selenium

Accepted for publication in Aquaculture Research
(DOI: 10.1111/are.13444)

8.1. Introduction

Barramundi (*Lates calcarifer*) is a commercial aquaculture species in Asia Pacific region and Australia. According to FAO (2016), world aquaculture production of barramundi has, due to intensive farming system, grown considerably over the last half-decade to be 75,374 tonnes in 2013. Thailand, Indonesia, Malaysia and China were the major producers (FAO, 2016). However, as with the farming of other carnivorous marine finfish species, potential damage to marine resources through high reliance on fishmeal (FM) as the major input of protein may further threatens the sustainability of barramundi aquaculture (Tacon & Metian, 2008). Therefore, many scientific investigations representing a variety of ingredients, diet formulation and experimental layouts have been conducted to study the incorporation of a non-FM dietary ingredient derived from plant protein (PP) sources, which are considered to be economically and ecologically sustainable.

Lupin meal (LM) has gained significant attention as a potential substitute to FM owing to the balanced nutritional properties, desirable palatability, high digestibility, inexpensive price and reliable supply. When fed to barramundi, digestibility of LM was high (Tabrett et al., 2012) and 45% LM protein inclusion in the diets was possible without impairing growth performance and protein metabolism (Katersky & Carter, 2009). Lactobacillus-fermented LM replacing 60% FM protein improved the performance of barramundi, though the protein retention in FM was higher than in LM diets (Van Vo et al., 2015). These findings suggest that barramundi appear to have a high tolerance for PP sources, particularly LM, which agrees with previous work of Glencross et al. (2011) that proposed a threshold level of FM being 15% of the diets.

However, a major hindrance of using PP ingredients as component of aquaculture feeds is the presence of antinutritional factors (ANF), such as phytate, which may induce morphological and physiological problem, thus affecting overall fish growth. Phytate, also known as myo-inositol hexakisphosphate or IP6, is the principal storage form of both phosphorus (P) and inositol in plant tissues (Salim ur et al., 2014). Because fish lack the digestive enzyme needed to disintegrate P from the phytate molecule, digestibility of phytate by fish is generally poor (Kumar et al., 2012). In addition, phytate can interfere with protein utilisation by forming phytate-protein complexes that may diminish the functions of digestive enzymes (Selle et al., 2012). Further, phytate has also been demonstrated to complex with minerals such as zinc (Zn), nickel (Ni), manganese (Mn), iron (Fe), magnesium (Mg), calcium (Ca) and selenium (Se) (Connelly, 2011 ; Reddy et al., 1989), reducing the bioavailability of these minerals for absorption. As reviewed by Kumar et al. (2012) and Prabhu et al. (2014), it is generally agreed that trace mineral is poorly bioavailable in diets containing high phytate levels. For this reason, attempts to formulate nutritionally-improved PP-based diets with decreased phytate level and thus to enhance trace mineral bioavailability remains seminal.

One possible technique is exogenous supplementation of trace minerals; of those, selenium (Se) is gaining a great deal of interest in animal feed either at research-scale or at commercial-scale application. Se is an important trace element required for normal life processes (Watanabe et al., 1997). Se is responsible for skeletal development (Lemly, 2002), antioxidant enzymatic function (Ashouri et al., 2015 ; Zhou et al., 2009) and proper immune system (Le et al., 2014b ; Wang et al., 2013). Se's underlying role is as an element of the antioxidant enzyme glutathione peroxidase (GPx) which protects cell membranes at both cellular and subcellular levels against oxidative damage by eliminating strong prooxidants such as hydroperoxides (Rotruck et al., 1973). Fish may obtain Se from the food and also from surrounding water, however dietary exposure to Se compounds is the predominant source of Se for fish (Janz, 2011). Therefore, the activity of GPx can be modified by dietary Se level. Furthermore, organic Se (OS) has been reported to be more bioavailable than inorganic Se for fish (Bell & Cowey, 1989 ; Le & Fotedar, 2014a ; Wang & Lovell, 1997). Selenoamino acids are the major form of OS, with selenomethionine (Se-Met) representing about half of the total Se (Lyons et al.,

2007). Trace element amino acids chelates have been widely applied in terrestrial and aquatic animal nutrition, yet very limited information is available on supplementation effect of OS in PP-based diets.

Another possible method to improve the quality of PP sources is by fermentation. A solid state fermentation process has been reported to destroy phytate and tannins present in LM (Van Vo et al., 2015). The degradation of ANF components such as phytic acid, trypsin inhibitor and lectin from soybean meal (SBM) was reported after 12 days of incubation with *Candida utilis* (Zhou et al., 2011). In addition, the fermentation of LM and SBM with lactic acid bacteria was reported to increase nutrient digestibility (Bartkiene et al., 2015), such as in grouper (*Epinephelus coioides)* (Zhuo et al., 2014). Through fermentation with *Bacillus subtilis*, phytate and free gossypol from cottonseed meal (CSM) were reduced by 88% and 60%, respectively (Sun et al., 2015a). Nutritive value of PP products could also be enhanced by fermentation with baker's yeast *Saccharomyces cerevisiae*, as reported by Hassaan et al. (2015).

However, based on the above highlight, an integrated approach that combines trace mineral supplementation and fermentation seems to be the best available strategy to enhance the content and bioavailability of trace elements in PP-based diets in sustainable aquaculture settings. Our recent study suggested that LM-derived phytate might have constrained Se bioavailability and thus promote adverse performance in juvenile barramundi (Ilham et al., 2016a). It is thus expected that juvenile barramundi fed with LM should perform as well as fish fed with FM diets as the sole protein source provided trace mineral bioavailability of the diets are simultaneously enhanced. Therefore, an attempt was designed to assess the efficacy of dietary OS supplementation in diets containing fermented LM replacing 75% FM protein on growth performance, enzymatic activity of GPx and biochemical status of juvenile barramundi (*L. calcarifer*).

8.2. Materials and methods

The feeding experiment was performed at the Curtin Aquatic Research Laboratory (CARL), Curtin University, WA, Australia. All the procedures were conducted in accordance with the Australian Code of Practice for the care and use of animals for

scientific purposes. The methodology of this experiment was approved by the Animal Ethics Committee of Curtin University (Approval Number: AEC-2013-07).

8.2.1 Preparation of fermented LM

LM were powdered and sieved through a 0.5-mm sieve and used as the raw material for fermentation. The condition of solid state fermentation follows a technique as described by Hassaan et al. (2015), with some modifications. Briefly, 2 kg LM and 66 mg of baker's yeast, *Saccharomyces cerevisiae* were added with 1.6 L of distilled water and homogenized in a food mixer for 15 min. The mixture was placed in a rectangular glass container covered with aluminium foil and incubated at 30ºC for 5 days. Then the product of fermentation was dried at 60ºC for 24 h and used as an ingredient for feed preparation. A representative sample was taken for amino acids (AA) and phytate content analysis of the ingredients as presented in Table 8.1.

Table 8.1. AA (g 100 g^{-1} protein) and phytate composition (%) of FM, LM and fermented LM

	FM	LM	Fermented LM
Arginine	4.35	4.67	4.80
Histidine	2.09	1.11	1.24
Isoleucine	3.04	1.80	2.63
Leucine	5.17	2.90	3.85
Lysine	4.77	1.84	2.81
Methionine	1.86	0.31	0.91
Phenylalanine	2.87	1.78	2.66
Threonine	3.19	1.58	2.17
Valine	3.26	1.70	2.42
Alanine	4.42	1.52	2.12
Aspartic acid	6.20	4.15	4.97
Glutamic acid	8.25	8.84	9.93
Glycine	4.73	1.75	1.98
Proline	3.81	2.12	4.21
Serine	3.05	2.14	2.89
Phytic acid (%)	–	0.61	0.18

8.2.2 Experimental diet

All feed ingredients were used in the present study were commercially obtained from Specialty Feeds (Glen Forrest, WA, Australia). Four isonitrogenous and isocaloric diets were formulated to contain 49% crude protein and 20 MJ kg^{-1} gross energy. LM replacing 75% FM protein either fermented or non-fermented (FLM and LM, respectively) and supplemented with 2 g OS kg^{-1} (FLM_{OS} and LM_{OS}, respectively). A FM-based diet formulated for barramundi according to known nutritional requirements (Catacutan & Coloso, 1995 ; Catacutan & Coloso, 1997 ; Glencross, 2006) was used as the control diet. The composition of the experimental diets is presented in Table 8.2.

Experimental diets were prepared according to standard CARL methods. All dry ingredients were thoroughly ground and sieved through a 0.5 mm mesh screen, mixed manually and transferred to a food mixer for 15 min. 5 g kg^{-1} chromic oxide (Cr_2O_3, Thermo Fisher Scientific, Scoresby, Vic., Australia), which was used as an inert indicator for digestibility measurement, was dissolved in 100 mL of distilled water and sprayed on the mash during mixing. Fish oil was dispersed and distilled water were added to the pre-mixed ingredients and mixed for another 15 min, yielding stiff dough. The mixture was then pelleted using a laboratory pelleting machine to the desired size and air-dried. 50 g of each diet was sampled for proximate composition and Se analysis of the diets as shown in Table 8.2. The experimental diets were stored at 4°C until use.

8.2.3 Fish and experimental condition

Juvenile barramundi were sourced from the Australian Centre for Applied Aquaculture Research (ACAAR), Fremantle, Western Australia (WA), Australia. Fish were acclimated to laboratory conditions for one week prior to the commencement of the trial, and all fish were fed the commercial diet twice daily during this period. Fish were then starved for 24 h, bulk-weighed, and a total of 300 healthy and homogenous sized juveniles (average individual weight of 5.88 ± 0.18 g) were randomly distributed among fifteen 300-L experimental tanks at stocking density of 20 fish $tank^{-1}$. Each of the experimental diets was randomly assigned to triplicate groups. All fish were hand-fed the respective experimental diets and the control diet to apparent satiation twice daily at 0900 h and 1500 h, 7 days a week for

75 days. Every 15 days, fish were weighed in bulk to calculate growth. Dead fish were weighed and recorded to adjust the calculation of the feed conversion ratio (FCR) and survival.

Table 8.2. Formulation and proximate composition of experimental diets for juvenile barramundi

Ingredient[a] (g kg^{-1} DM basis)	Diets[b]				
	LM	LM_{OS}	FLM	FLM_{OS}	FM
FM	150	150	180	180	460
LM[c]	510	510	450	450	–
Wheat gluten	100	100	100	100	100
Wheat flour	–	–	–	–	80
Casein	120	120	120	120	120
Fish oil	80	80	90	90	100
Wheat starch	15	13	20	20	65
Cellulose	–	–	15	13	50
Se-free premix[d]	20	20	20	20	20
Organic selenium[e]	–	2	–	2	–
Chromic oxide	5	5	5	5	5
Proximate composition					
Dry matter	90.17	90.78	88.72	88.75	85.17
Ash	5.37	5.33	6.51	6.58	8.25
Crude protein	48.21	48.30	48.67	48.71	49.07
Crude lipid	14.50	14.52	14.65	14.66	15.06
Gross energy	20.65	20.57	20.43	20.42	21.33
Se (mg kg^{-1})	1.53	3.52	1.84	3.81	3.17

[a] Supplied by Specialty Feeds, Perth, WA, except for Sel-Plex and chromic oxide, obtained from AllTech, Lexington, Kentucky, USA and Thermo Fisher Scientific, Scoresby, Vic., Australia, respectively.

[b] LM (lupin meal); LM_{OS} (LM+Se); FLM (fermented LM); FLM_{OS} (fermented LM+Se); FM (fishmeal)

[c] Australian sweet lupin, *Lupinus angustofolius*

[d] Contains the following (as g kg^{-1} of premix): iron, 10; copper, 1.5; iodine, 0.15; manganese, 9.5; zinc, 25; vitamin A retinol, 100 IU; vitamin D3, 100 IU; vitamin E, 6.25; vitamin K, 1.6; vitamin B1, 1; vitamin B2, 2.5; niacin, 20; vitamin B6, 1.5; calcium, 5.5; biotin, 0.1; folic acid, 0.4; inositol, 60; vitamin B12, 0.002; choline, 150; ethoxyquin, 0.125

[e] Sel-Plex®

During the experimental period, the tanks were supplied with recirculated water from an external biofilter (Fluval 406, Hagen, Italy) at a flow rate of 10 L min^{-1}. All tanks were supplied with continuous aeration and pure oxygen (compressed oxygen, BOC, Perth, WA). One-third of the water was exchanged every two weeks during the rearing period. Water quality parameters such as temperature, dissolved oxygen, and salinity were measured daily and maintained at 28-29 ºC, >5 mg L^{-1}, 32-34 ppt, respectively. These values are considered within optimal ranges for juvenile barramundi. The light-dark regime was maintained at 12:12 h per day.

8.2.4 Digestibility assessment

The digestibility of the diets was determined by total collection of faecal matter, for which faecal collections from individuals were pooled by tank, over a 7-day span prior to the end of the feeding experiment. The faecal was manually stripped immediately before the morning feeding by applying pressure the lower abdominal region of the fish. Care to exclude urine, mucus or water from the faecal samples was applied (Austreng, 1978 ; Glencross et al., 2005). Apparent digestibility coefficient (ADC) of dry matter (DM), protein and lipid for experimental and control diets was calculated according to the method of Maynard and Loosli (1969).

8.2.5 Sampling and chemical analysis

After the feeding trial, all fish were deprived of food for 24 hours before sampling. The fish were then anaesthetised with tricaine methanesulfonate (MS-222, Sigma-Aldrich, Castle Hill, NSW, Australia) at 100 mg L^{-1} and weighed individually to compute the weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and survival. For the determination of haematological indices, blood was withdrawn from the caudal vein puncture of three randomly selected fish per tank (9 fish per dietary treatment). The extracted blood in a 1 mL plastic syringe was transferred BD Vacutainer® blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Subsamples of whole blood were immediately analysed for haemoglobin (Hb), haematocrit and leucocrit concentrations. Remaining blood samples were then centrifuged for 5 min at 12,000 rpm at 4 ºC, and the plasma samples collected were stored at 4 ºC until subsequent blood chemistry analyses were

performed. Enzyme activity of GPx was quantitatively measured by using the Randox Laboratories test combination (Ransel, Antrim, United Kingdom).

Blood samples were sent to the Animal Health Laboratories (South Perth, Western Australia) for plasma enzyme and biochemistry analysis. The assays were run on an Olympus AU400 automated chemistry analyser (Olympus Optical Co. Ltd, Mt Waverley, VIC, Australia). Each of the assays used was a standard kit developed for the auto-analyser. The tests performed included creatinine kinase (CK) (Olympus kit Cat. No. OSR6179), alanine aminotransferase (ALT) (Olympus kit Cat. No. OSR6107), urea (Olympus kit Cat. No. OSR6134), creatinine (Olympus kit Cat. No. OSR6178), phosphate (Olympus kit Cat. No. OSR6122), cholesterol (Olympus kit Cat. No. OSR6116), total protein (Olympus kit Cat. No. OSR6132), and albumin (Olympus kit Cat. No. OSR6102). Haptoglobin was measured based on method as described by Eckersall *et al.* (1999).

The proximate composition of the diet and faecal samples were determined in triplicate based on standard procedures (AOAC, 1990). Briefly, dry matter was calculated by gravimetric analysis following oven drying to constant weight at 105ºC. Crude ash was determined by combustion in a furnace at 550 ºC. Crude protein content was determined by measuring nitrogen (N × 6.25) using the Kjeldahl digestion method. Lipid content was determined by ether extraction using Soxhlet technique. Gross energy content was measured by an IKA oxygen bomb calorimeter (Heitersheim, Germany). AA content of the ingredients was quantified after samples were hydrolysed in HCl (Barkholt & Jensen, 1989 ; Rayner, 1985). Analyses were performed on an Agilent 1100 series high-performance liquid chromatography (HPLC, Agilent Technologies, Germany) system using conditions similar to those described by Gratzfeld-Huesgen (1998). Phytate content of the ingredients was determined using automated high-performance liquid chromatography (HPLC) analysis as suggested by Kwanyuen and Burton (2005).

8.2.6 Se determination

Se was analysed at the Intertek Genalysis Laboratory (Perth, Australia) using inductively coupled plasma-mass spectrometry instrument (ICP-MS, 7500 series, Agilent Technologies, Australia), as previously described in detail (Ilham et al., 2016a). Se concentration was measured as dry weight.

8.2.7 Calculations

Growth, feeding utilisation and ADC were measured using the calculated parameters as follows:

$$\text{Specific growth rate (SGR, \% day}^{-1}\text{)} = 100 \times \left[\frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{days}}\right]$$

$$\text{Weight gain (\%)} = 100 \times \left[\frac{\text{final weight-initial weight}}{\text{initial weight}}\right]$$

$$\text{Feed intake (FI, g fish}^{-1}\text{ days}^{-1}\text{)} = \left[\frac{\text{dry diet given} - \text{dry remaining diet recovered}}{\text{number of fish}}\right] / \text{days}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake}}{\text{weight gain}}$$

$$\text{Survival (S, \%)} = 100 \times \left[\frac{\text{final number of fish}}{\text{initial number of fish}}\right]$$

$$\text{ADC (\%)} = 100 - \left[(100 - \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{protein in faeces}}{\%\ \text{protein in diet}})\right]$$

8.2.8 Statistical analysis

All statistical analyses were performed using SPSS (version 22, IBM Inc., Australia). Data regarding the effects of LM product, Se level and their interactions on growth, feeding, digestibility, enzymatic activity, haematological and biochemical status were subjected to two-way analysis of variance (ANOVA). Assumptions of homogeneity of variances were checked using Levene's equal variance test. One-way ANOVA was performed to compare the control FM diet against each experimental diet. When significant effects were obtained for a factor, the Duncan test was used to compare the reference diet against each test diet (FM *versus* LM, LM_{OS}, FLM, and FLM_{OS}). Means were considered to be significantly different when $P < 0.05$. To meet ANOVA requirements, percentage data such as survival and ADC were computed using arcsine square root transformation.

8.3. Results

8.3.1 Growth performances

Final survival, growth performance and feed utilisation of *L. calcarifer* fed diets containing fermented lupin meal and OS for 75 days are shown in Table 8.3. No

interaction between fermentation and OS level was found to affect survival, growth and feeding performances. Instead, at the termination of the feeding experiment, the survival of juvenile barramundi in all dietary treatments was not significantly different, ranging between 95–100% ($P > 0.05$). Meanwhile, FW, SGR, WG and FCR of fish were influenced by the type of LM diets ($P < 0.05$). During the feeding trial, fish grew from 5.88 g to an average of 39.11 g in non-fermented dietary groups and 43.52 g in fermented dietary groups. SGR varied between 2.72% and 2.92% body weight day^{-1} among the dietary groups. Fish fed with fermented LM diets grew significantly faster and weighed substantially more than fish fed non-fermented LM diets. Fish fed with diets containing fermented LM diets were observed to gain better FCR compared to fish fed with the non-fermented LM diets ($P < 0.05$). However, the FW, SGR and FCR of fish fed FLM and FLM_{OS} diets were similar to those of fish fed the control FM diets ($P > 0.05$).

8.3.2 Digestibility

As presented in Table 8.4, fermentation of LM increased ADC of DM similar to FM diets ($P > 0.05$). The ADC of protein was affected by both LM type and OS level ($P < 0.05$). Fish fed diets containing fermented LM attained higher ADC of protein compared to those fed non-fermented LM. Likewise, OS supplementation in the diets increased the ADC of protein by around 3%. However, the ADC of lipid was affected neither by LM type and OS level, nor by their interaction ($P > 0.05$).

Table 8.3. Survival, growth performance and feed utilisation of juvenile barramundi fed test diets formulated with lupin meal (LM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	LM	LM_{OS}	FLM	FLM_{OS}	SEM	LM	OS	LM×OS	
Survival	95	95	100	97	1.058	0.776	0.127	0.495	100
FW	38.63*	40.19*	43.19	43.86	0.481	0.001	0.265	0.844	44.25
SGR	2.72*	2.77*	2.88	2.92	0.025	0.003	0.148	0.378	2.93
WG	667.9*	678.92*	716.8	757.7	0.015	0.002	0.147	0.363	798.5
FI	0.63	0.61	0.62	0.62	0.011	0.916	0.469	0.916	0.65
FCR	1.39*	1.33*	1.27	1.25	0.045	0.044	0.296	0.885	1.28

Two-way ANOVA performed involving only the experimental diets.
ns not significant; SEM pooled standard error of the mean.
Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$)
FW (final weight, g); SGR (specific growth rate, % body weight day^{-1}); FI (feed intake, g $fish^{-1}$ day^{-1}); WG (weight gain, %); FCR (feed conversion ratio); S (survival, %).

Table 8.4. Apparent digestibility coefficients (ADC) of dry matter, protein and lipid of juvenile barramundi fed test diets formulated with lupin meal (LM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	LM	LM_{OS}	FLM	FLM_{OS}	SEM	LM	OS	LM×OS	
Dry matter	83.1*	83.2*	88.4	88.3	0.692	0.006	0.907	0.564	89.3
Protein	91.3*	93.5	95.1**	96.2**	0.513	0.001	0.036	0.596	93.5
Lipid	94.7	94.5	96.2	95.5	0.664	0.071	0.942	0.237	96.1

Two-way ANOVA performed involving only the experimental diets.
ns not significant; SEM pooled standard error of the mean.
Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$).

8.3.3. Muscle Se level

At the end of feeding trial, muscle Se concentration was significantly influenced by OS supplementation (Figure 8.1). The Se level in the muscle of juvenile barramundi fed with OS-supplemented diets was higher than that of fish fed with other diets ($P < 0.05$). In addition, there was no significant differences in the muscle Se concentration between fish fed with fermented LM-based diets and those fed with LM-based diets without fermentation ($P > 0.05$). When fish fed with FM-based diets, the muscle Se level was similar to those fed FLM diets, but lower than those fed with OS-supplemented diets.

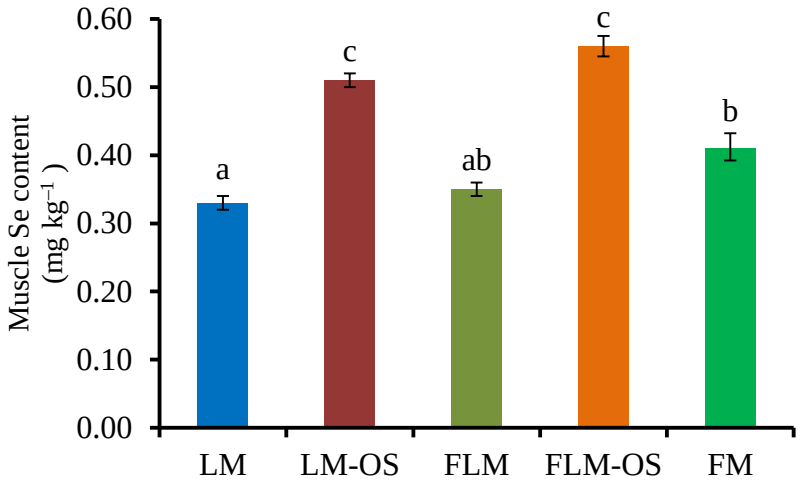

Figure 8.1. Muscle Se content of juvenile barramundi fed experimental diets over 75 days.

8.3.4. GPx activity and haematology

GPx activity of the experimental fish is shown in Figure 8.2. Fish fed diets supplemented with OS attained higher GPx activity than those fed diets without OS supplementation. In the same way, fish fed diets containing fermented LM had significantly higher GPx activity than fish fed non-fermented LM diets. The GPx activity of fish fed the control diets was similar to that of fish fed the FLM and LM_{OS} diets, but lower than FLM_{OS} diets.

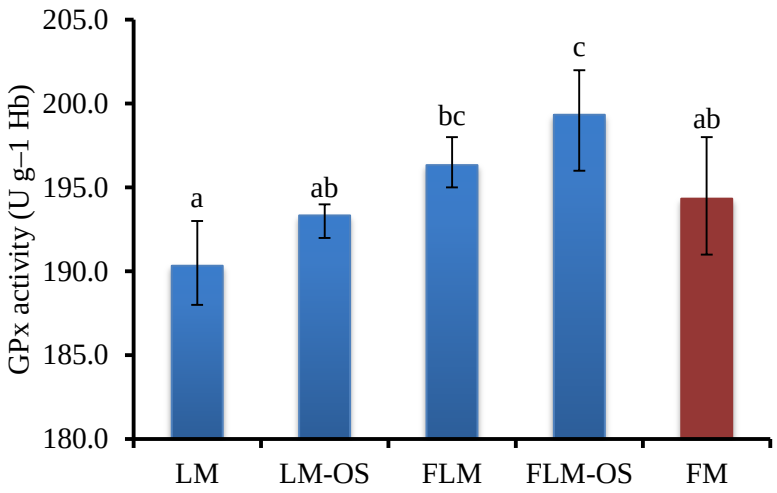

Figure 8.2. GPx activity of juvenile barramundi fed experimental diets over 75 days.

As displayed in Table 5, the highest levels of Hb were in fish fed fermented LM diets ($P < 0.05$). However, these levels were not significantly different from that of fish fed the control diets ($P > 0.05$). No significant differences in haematocrit were shown among dietary groups ($P > 0.05$). Leucocrit levels of fish fed the fermented LM and the control FM diets were similar ($P > 0.05$) and significantly higher than that of fish fed non-fermented LM diets ($P < 0.05$). Meanwhile, GPx activity was significantly influenced by both fermentation and OS supplementation.

Table 8.5. Haematological indicator and GPx of juvenile barramundi fed test diets formulated with soybean meal (LM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	LM	LM_{OS}	FLM	FLM_{OS}	SEM	LM	OS	LM×OS	
Hb	72	73	75**	75**	0.553	0.001	0.223	0.493	73
Haematocrit	32.3	34.7	35.7	35.4	0.648	0.056	0.300	0.170	34.8
Leucocrit	1.08*	1.23*	1.37	1.44	0.076	0.011	0.194	0.600	1.47

Two-way ANOVA performed involving only the experimental diets.
ns not significant; SEM pooled standard error of the mean.
Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$).
Hb (haemoglobin, g dL^{-1}); haematocrit (%); leucocrit (%).

8.3.5 Biochemical status

Blood biochemistry of the experimental fish is presented in Table 8.6. The highest total protein (TP) was found in fish fed fermented LM diets. The TP plasmatic concentrations between non-fermented LM and the control FM diets were similar ($P > 0.05$), which was around 40–43 g L^{-1}. There was an effect in plasma albumin concentration in the dietary treatments. With fermentation of LM, albumin level was higher than in fish fed diets containing non-fermented LM. However, the A/G ratio was not affected either by fermentation and OS supplementation. Furthermore, plasma ALT activity was significantly higher in fish fed non-fermented diets (LM and LM_{OS}) than in those fed fermented LM diets (FLM and FLM_{OS}) ($P < 0.05$). However, there were no significant differences in ALT activity among LM_{OS}, FLM, FLM_{OS} and FM diets. There was an interactive effect between the LM and OS on plasma CK concentration. The CK of fish fed diets supplemented with OS was higher in non-fermented LM diets but lower in fermented LM diets ($P < 0.05$). In addition, both fermentation and OS supplementation level affected the CK plasmatic concentration ($P < 0.05$). CK concentration of fish fed fermented LM diets was lower than that of fish fed non-fermented diets. Also, OS-supplemented diets were able to reduce the CK levels of fish. No significant differences were observed in haptoglobin, urea, creatinine, phosphate and cholesterol among the dietary treatments ($P > 0.05$).

Table 8.6. Plasma biochemistry contents of juvenile barramundi fed test diets formulated with soybean meal (LM) and simultaneous supplementation with OS for 75 days

Parameter	Diet					Two-way ANOVA			FM
	LM	LM_{OS}	FLM	FLM_{OS}	SEM	LM	OS	LM×OS	
Total protein	40	43	45**	45**	1.130	0.029	0.402	0.402	41
Albumin	13*	15	16	16	0.726	0.018	0.107	0.147	16
A/G ratio	0.6	0.6	0.7	0.7	0.024	0.071	0.084	0.506	0.6
Haptoglobin	0.18	0.17	0.17	0.16	0.009	0.434	0.598	0.908	0.15
Urea	9	9	10	9	0.687	0.488	0.260	0.500	8
Creatinine	20	21	18	19	1.130	0.272	0.402	0.998	20
Phosphate	3.3	3.6	3.5	4.1	0.421	0.675	0.731	0.203	3.5
Cholesterol	7.1	6.3	6.4	6.5	0.232	0.264	0.175	0.085	7.4
ALT	18**	15	14	14	1.167	0.046	0.235	0.100	13
CK	3380**	2892	2723	2767	47.237	0.001	0.033	0.012	2788

Two-way ANOVA performed involving only the experimental diets.

ns not significant; SEM pooled standard error of the mean.

Values in the same column with * and ** indicated significant differences against FM (** > FM < * when $P < 0.05$).

TP (total protein, g L^{-1}); albumin (g L^{-1}); A/G (albumin to globulin); urea (mmol L^{-1}); creatinine (μmol L^{-1}); phosphate (mmol L^{-1}); cholesterol (mmol L^{-1}); haptoglobin (mg L^{-1}); ALT (alanine aminotransferase, U L^{-1}); CK (creatinine kinase, U L^{-1}).

8.4. Discussion

The previous study suggested that 75% of FM protein can be replaced by LM with OS supplementation for the diets of juvenile barramundi (Ilham et al., 2016a). Thus, the present study was designed to investigate the effects of fermentation of LM simultaneously supplemented with OS on growth, feeding, enzymatic activity and biochemical status of the fish. Fermentation of LM (FLM and FLM_{OS}) significantly improved growth performances and FCR over the 75-day period. SGR observed in barramundi in the present study is comparable to data of our previous study (Ilham et al., 2016a). The works of Van Vo (2015) on similar species and Molina-Poveda (2013) on juvenile whiteleg shrimp (*Litopenaeus vannamei*) led to similar results. PP products resulted from microbial fermentation have been shown to successfully replace high levels of FM in the diets of grouper (*E. coioides*) (Shiu et al., 2015a) and whiteleg shrimp (*L. vannamei*) (Shiu et al., 2015b). Hassaan *et al.* (2015) found an improved growth performance of Nile tilapia (*Oreochromis niloticus*) when 50% FM protein was replaced by yeast-fermented SBM. In this study, high replacement of FM with fermented LM resulted in favourable growth performance, similar to that in fish fed the control FM diet.

Fermentation allows higher substitution level of FM with plant-derived proteins through deactivation of ANFs, formation of low molecular weight protein and improvement of protein digestibility (Hotz & Gibson, 2007). Commercial strain of baker's yeast *S. cerevisiaei* have been used for the production of microbial phytase (Caputo et al., 2015), an enzyme that plays a well-characterised role in the hydrolysis of antinutritional phytate (Kumar et al., 2012). In the present study, a significant reduction in phytate content (70.52%) in yeast-fermented LM was found, indicating that the phytase activity of the baker's yeast was boosted during fermentation process. This agrees with earlier findings that fermentation of PP sources with *S. cerevisiaei* can degrade phytic acid to below unfavourable level (Tudor et al., 2013), which may offer nutritional advantages to monogastric animals such as fish. Sharawy *et al.* (2016) reported that solid state fermentation of PP feedstuffs with *S. cerevisiaei* is able to reduce phytate and trypsin inhibitor concentrations and thus increase the inclusion level of plant-derived protein up to 50% in the diets of Indian white shrimp (*Fenneropenaeus indicus*). In addition, Belewu and Sam (2010) examined the effect of solid state fermentation *Jatropha curcas* kernel cake by various fungi on ANFs

and reported a dramatic decrease in phytate, lectin, trypsin inhibitors and saponin concentrations in fermented *Jatropha curcas* kernel cake. In the present work, fermented LM-fed fish outperformed those fed the non-fermented LM diets, suggesting that the superior growth promoting capacity of the fermented LM diets may have been partly due to decreased antinutritional content.

The major alteration during the fermentation process includes the breakdown of proteins into peptides and AA and low molecular weight compounds (Bartkiene et al., 2015 ; Zhuo et al., 2014). Increased AA contents could be indicative of the refinement of LM product as achieved in the present study. Overall, after fermentation, AA content of the essential and non-essential increased by 52.08% and 36.39%, respectively. The AAs predominantly credited for this increase were methionine, proline and lysine followed by phenylalanine, isoleucine, valine, alanine, threonine, serine, leucine, aspartic acid, glycine, glutamic acid, histidine and arginine. It is essential that the AA content of fermented LM echoes a balanced dynamic equilibrium, which might have been induced by proteolytic activities during fermentation. Increased AA content in solid state fermentation of various PP ingredients has also been reported in earlier studies with LM (Van Vo et al., 2015), SBM (Gao et al., 2013 ; Hassaan et al., 2015 ; Shimeno et al., 1993), and CSM (Lim & Lee, 2011 ; Sun et al., 2015b). Increased crude protein (29.78%) observed in the present study were attributable to increases in overall AA content, as demonstrated by Shiu *et al.* (2015b). Accordingly, improvement of crude protein might lead to reduced amounts of fermented LM being incorporated in fish diets.

The present study indicated that, although barramundi seemed to tolerate high inclusion level of LM in diets based on ADC observed in our previous study (Ilham et al., 2016a), high amounts of non-fermented LM in the diets deleteriously affect FCR, more so than fermented LM and FM. Poor FCR have been recorded in Nile tilapia (*O. niloticus*) fed non-fermented PP sources than in those fed fermented feeds (Lim & Lee, 2011). In constrast, inclusion of 400 g kg^{-1} fermented SBM in the diets of pompano (*Trachinotus ovatus*) resulted in higher FCR and poor growth performance (Lin et al., 2012). In fish, unsatisfactorily nutrient intake can affect overall metabolism, thus it might be that energy required to maintain this metabolism would have reduced the available energy for growth and decrease FCR.

The results of this study revealed that high inclusion level of LM decreased the ADC of DM, which seemed to be associated with phytate level, as found in previous studies (Baruah et al., 2007 ; Plaipetch & Yakupitiyage, 2014 ; Zhou et al., 2011). Therefore, fermentation appears to confer important benefits to the improvement of ADC of DM, through degradation of phytate contained by PP sources. Zhuo *et al.* (2014) reported ADC of DM and protein of fermented SBM in excess of 83% and 93%, respectively, for grouper (*E. coioides*). In comparison, Ng *et al.* (2002) showed that, without fermentation, poor nutrient digestibility values were obtained from palm kernel meal by red hybrid tilapia (*Oreochromis* sp.). Further, it was observed that juvenile barramundi efficiently utilised protein from the fermented LM diets (FLM and FLM_{OS}). The ADCs of protein were substantially higher for fermented LM diet than for non-fermented LM and FM control diet. The findings of the present study agrees with earlier investigation by Zhou and Yue (2012) who found that fermented PP products improved the ADC of protein higher than that of FM.

Apparently, high protein digestibility of fermentation-treated PP-based diets can be related to increases in indispensable AA content as previously explained and reduction in phytate content. A progressive increase in protein digestibility with decreased level of phytate has been recorded in FM replacement studies on various fish species (Baruah et al., 2007 ; Hussain et al., 2015 ; Mwachireya et al., 1999 ; Storebakken et al., 1998). Interestingly, despite the fact that digestibility of LM is low on account of its high fibre content (Glencross, 2009), the ADC of DM and protein in the present study were higher than the findings reported by previous studies (Borquez et al., 2011 ; Farhangi & Carter, 2007 ; Ilham et al., 2016a ; Molina-Poveda et al., 2013), likely due to an amelioration effect of the fermentation process.

Regardless of fermentation, the ADC of protein in fish fed OS-supplemented diets was significantly higher than that of fish fed diets without OS supplementation and FM control diet. When fed to juvenile barramundi, the FLM_{OS} diet gave the highest ADC value. Therefore, these results suggest that both fermentation and OS supplementation plays an important role in protein metabolism. However, the effect of OS supplementation on the ADC of protein did not lead to improvement in the growth performance of the fish.

Although there was a slight reduction in SGR of juvenile barramundi fed diets without OS supplementation, growth performance of the fish was not affected by dietary OS supplementation as reported for rainbow trout (*Oncorhynchus mykiss*) (Hilton et al., 1980).

Previously, PP-based diets deficient in Se were acceptable for growth although supplemental Se was required to maintain optimal GPx activity of rainbow trout (*O. mykiss*) (Fontagne-Dicharry et al., 2015). Additionally, in a FM-based study, supplemental Se was not required to enhance growth although it seemed to be necessary for GPx activity maintenance in common carp (*Cyprinus carpio*) (Elia et al., 2011).

In contrast, a FM-based diet lacking Se supplementation resulted in depressed growth and reduced GPx activity when fed to channel catfish (*Ictalurus punctatus*) (Gatlin & Wilson, 1984). Similar results have been found for grouper ((*E. coioides)* (Lin & Shiau, 2005), cobia (*Rachycentron canadum*) (Liu et al., 2010), gibel carp (*Carassius auratus gibelio*) (Han et al., 2011), largemouth bass (*Micropterus salmoide)* (Zhu et al., 2012), and yellowtail kingfish (*S. lalandi*) (Ilham & Fotedar, 2016 ; Le & Fotedar, 2013b). Moreover, our previous studies also suggested that appropriate level of dietary Se was needed to support growth and antioxidant GPx activity of barramundi when fed with diets containing high inclusion level of PP sources such as LM and SBM (Ilham et al., 2016a ; Ilham et al., 2016c).

However, Prabhu *et al.* (2014) conducted a systematic review on mineral requirements of fish and concluded that, when WG is used as the response criterion, dietary Se affect GPx activity rather than growth, thus GPx activity comprise a more robust marker of the bioavailability and utilisation of dietary Se in fish. In the present study, FLM_{OS} diet (3.81 mg Se kg^{-1}) generated the highest GPx activity level of juvenile barramundi, indicating that combined strategy of Se supplementation and fermentation promotes beneficial effects in enhancing the antioxidant capacity of fish. In addition, the muscle tissue Se levels of fish fed with the LM_{OS} and FLM_{OS} diets were higher than that of fish fed with the other diets, indicating that trace mineral (Se) utilisation was improved by OS supplementation. Similarly, Se contents in juvenile yellowtail kingfish (*S. lalandi*) were markedly increased with the supplementation of dietary OS (Ilham et al., 2016b).

As some minerals such as P, Zn and Se are known to be less bioavailable in plant-derived protein sources (Prabhu et al., 2014), the decline in FM use in aquaculture feeds might result in the adjustment of those minerals supply to fish. The presence of ANF in PP-based diets can be of nutritional significance, particularly with the aforementioned minerals. For instance, phytate in plant feedstuffs interferes with the absorption of trace minerals and diminish their bioavailability (Francis et al., 2001). Storebakken *et al*. (1998) demonstrated that inclusion of soy concentrate with high phytate concentration (18 g kg^{-1}) in the diets of Atlantic salmon (*Salmo salar*) led to reduction of bioavailable P, Ca, Mg and Zn. Sajjadi and Carter (2004) reported that P digestibility and retention efficiency were reduced when Atlantic salmon (*S. salar*) was fed with phytate-containing canola meal-based diet. SGR of mrigal (*Cirrhinus mrigala*) was significantly reduced when phytate level exceeded 1% of total diet (Usmani & Jafri, 2002). In addition, decline in WG, FCR and Zn content in the vertebrae of channel catfish (*I. punctatus*) was related to the elevation of phytate level (2.2%) in the diet (Satoh et al., 1989). Therefore, with Se, exogenous supplementation of Se to meet the Se requirement of fish has been the major point of concern in recent years.

Apart from the abovementioned interaction of dietary sources with Se, particular nutrients exist in the diet may also interact with Se. Prabhu *et al.* (2014) described that interaction with a specific trace nutrient may influence the minimum dietary incorporation level of Se. The synergistic interaction between Se and vitamin E has been documented (Watanabe et al., 1997). Although Se and vitamin E have particularised roles, both nutrients are involved in the enhancement of cellular antioxidant protection system. While Se functions as component of enzymatic GPx, which devastates hydrogen peroxide and lipid hydroperoxides, vitamin E is a cellular-related antioxidant and free radicals scavenger, which protect biological membranes against lipid peroxidation (Combs & Combs, 1986). Accordingly, the mutual sparing effects of Se and vitamin E allow practical implication in aquaculture feed formulation (Lin & Shiau, 2009). Thus, synergism between Se and vitamin E when fish are fed high level of plant-derived protein sources merits further investigation.

Blood haematological indices of fish are known to be influenced by a variety of both internal and external factors including species, age, size, physiological circumstance, environmental conditions, and nutritional status (Soltanzadeh et al., 2015). Little information is available regarding the potential haematological effects of dietary PP intake in carnivorous marine finfish, particularly in barramundi. A well-defined linkage between FM substitution and haematological indicators has not been established in fish; nonetheless, inclusion of PP ingredients in the diet significantly modifies both qualitative and quantitative descriptions of Hb in great sturgeon (*Huso huso*) (Jahanbakhshi et al., 2013). In the present experiment, although OS supplementation did not affect the haematological status of fish as demonstrated in our previous study (Ilham et al., 2016a), dietary PP did. Fish fed diets containing fermented LM (FLM and FLM_{OS}) had higher Hb than those fed any other diets, suggesting a propitious utilisation of dietary fermented LM by juvenile barramundi, particularly in the maintenance of red blood cell functions. No published data were available with which to contrast the differences of Hb and leucocrit values in barramundi (*L. calcarifer*) reared on a fermented PP diet achieved in this study.

However, in beluga (*H. huso*), a decreasing trend was observed in the amounts of Hb when FM was replaced with PP sources (Jahanbakhshi et al., 2013). In addition, the Hb concentration obtained in the present study (75 g dL^{-1}) was comparable to that reported in healthy juvenile yellowtail kingfish (*S. lalandi*) (Le et al., 2014a). Further, leucocrit concentrations can indicate the health condition of fish (Wedemeyer et al., 1983), thus malnutrition and decreased resistance to disease are associated with low levels of leucocrit (El-Asely et al., 2014). In this study, significantly higher levels of leucocrit were observed in barramundi fed fermented LM diets, indicating that fermentation may induce specific compounds that promote leucocrit production. Adams *et al.* (1993) suggested that leucocrit assay is a quick and efficient tool for assessing fish health status.

Plasma TP was used as a fundamental indicator of physiological condition and health status of juvenile barramundi, in accordance with data reported for other species (Al-Dohail et al., 2009 ; Han et al., 2015 ; Katya et al., 2014 ; López et al., 2015 ; Sardar et al., 2007). The TP level for fermented LM-fed fish obtained in the present study (43 g L^{-1}) was slightly higher than the reported TP concentration for

barramundi when fed with narrowleaf lupin (*L. angustofolius*) kernel meal (43 g L^{-1}). TP is a key indicator of the metabolism of diet, and its deterioration might be affected by deprived nutrient digestion and metabolism (Soltanzadeh et al., 2015). Plasma proteins such as albumin is an essential fraction in the delivery of substances and in the enhancement of a vigorous immunological competence (Andreeva, 2012). Decreased plasmatic albumin was associated with inflammation processes as demonstrated in juvenile totoaba (*Totoaba macdonaldi*) (López et al., 2015). In the present study, it was observed that fish fed non-fermented, OS-unsupplemented diet (LM) had lowest albumin concentration, suggesting that the fish would have undergone inflammatory process in response to antinutrients harmful effects. Interestingly, the concentrations of TP and albumin in fish fed LM_{OS} diets were similar to that in fish fed the FM diet. Further research with longer duration is needed to evaluate whether the form of Se (i.e. organic *vs* inorganic) have the observable effect in barramundi with regard to plasmatic TP and albumin concentrations.

In fish, elevation of plasma enzymes is indicative of liver and muscle damage. While ALT activity plays a significant role as a biochemical indicator for liver health, CK activity is used as a biomarker enzyme for muscle injuries (Glencross et al., 2011). An elevation of ALT and CK activities has been reported in the plasma of barramundi when lupin kernel meal or wheat gluten was included in the diets (Glencross et al., 2011). Similarly, increased ALT in fish fed diets containing non-fermented LM was observed in the present study. However, no significant difference in ALT activity of the non-fermented LM diet supplemented with OS (LM_{OS}) relative to FM control diet, indicating that diet without fermentation and Se supplementation treatment (LM) might induce liver-associated syndromes. If this would be the case, the results of the present study corroborate the findings reported in our previous research in which lipid droplet congregation in hepatocytes was found when fish were fed diets containing high inclusion of LM (Ilham et al., 2016a). On the other hand, decreased ALT activity with increased inclusion level of faba bean (*Vicia faba*) was reported for beluga (*H. huso*) (Soltanzadeh et al., 2015). Lin and Luo (2011) also observed that there were no negative influences on liver enzymes when 50% or more SBM was replacing FM in hybrid tilapia (*O. niloticus* × *O. aureus*) diets.

Variations in ALT activity as demonstrated in these studies may reflect lack recognition in accordance with the biochemical roles of the ALT in fish (Soltanzadeh et al., 2015). However, as evident from the literatures cited hereby and the findings of the current experiment, the refinement of any PP ingredient is seen as a promising way to prevent fish from any lipotoxic damage in the liver. For instance, in starry flounders (*Platichthys stellatus*), inclusion of soy protein hydrolisates to replace 50% FM protein resulted in reduced ALT activity (Song et al., 2014). In line with this, in terrestrial animal, solid state fermentation of rapeseed meal has been reported to be able to maintain ALT activity at levels appropriate for pigs (Shi et al., 2015). Moreover, dietary PP ingredient refinement through fermentation with *S. cerevisiae* might improve liver functional performance for juvenile barramundi as demonstrated in this study.

Furthermore, plasma CK activity appeared to be influenced by fermentation and OS supplementation, and their interaction. Fermentation of LM was an important element responsible for reduced plasma CK activity in juvenile barramundi. Likely, for Se, fermentation enhanced functionality. As LM was fermented, the fish fed diets supplemented with OS was able to maintain the CK activity, similar to those fed the FM diet. Reduction of phytate concentration due to fermentation might have decreased the potential for phytate-Se complex formation, thus allowing better utilisation of LM to support overall growth and physiological properties. Further research examining the synergism between PP-based diet and trace mineral supplementation in fish is required. However, irrespective of the OS supplementation, feeding fish with fermented LM diets (FLM and FLM_{OS}) resulted in reduced amounts of plasma CK activity. These results indicated that fermentation with *S. cerevisiae* provided protection from the degeneration of muscle fibres, thus structurally improving the integrity of the muscle cells.

Previously, Glencross *et al*. (2011) observed that inclusion of LM (*L. angustofolius*) in diets of barramundi negatively affected the CK activity, achieving the level of 8,515 U L^{-1}. The authors also suggested that healthier fish maintained the CK activity at level around 3000 U L^{-1}, which was similar to the results in this study. In comparison, the reported normal values of CK activity for sturgeons (*Acipenser stellatus*) were 5,958–7,233 U L^{-1} (Shahsavani et al., 2008). The effects of variation

in species, nutritional status and environmental condition in the quantification of CK activity should be taken into account (Shi et al., 2006).

8.5. Summary

The findings of the present investigation reinforce an obvious potential to substitute FM with 75% fermented LM protein in the diets for barramundi (*L. calcarifer*) without triggering adverse impacts on growth and health performances. Fish fed diets containing fermented LM gained better growth, FCR, DM and protein ADC, which are attributable to the degradation of antinutritional phytate during *S. cerevisiae* fermentation process. In addition, the superiority of fish fed the FLM_{OS} diets as shown in the present study with regard to the enzymatic antioxidant GPx activity and plasma haemo-biochemical status might be due to proper utilisation of dietary nutrients in presence of supplemental OS.

CHAPTER 9: General discussion, conclusions and recommendations

9.1. General discussion

A bourgeoning demand for seafood consumption continues to accelerate growth in the aquaculture industry, at an annual average rate of 6% in the decade 2005-2014 (FAO, 2016). By 2020, roughly 120-130 million tonnes of fish are projected to be contributed by the aquaculture sector (Teves & Ragaza, 2016), thus, exaggerating pressure on aquaculture feed production, a key component of commercial aquaculture. Compound feed – feeds formulated from multiple ingredients – represents more than half of the total operational costs within aquaculture, with fishmeal (FM) predominantly used as a protein source due to its superior nutritional properties. While FM supplies have steadied, its utilisation in the diets of aquaculture species continues to grow (OECD/FAO, 2014 ; Olsen & Hasan, 2012).

Growing concerns around the sustainability of source fisheries and the increasing costs of FM offer a compelling impetus for the substitution of FM in aquaculture feed. Possible alternatives include plant protein (PP) ingredients and fish processing and agriculture industry by-products (Adelizi et al., 1998 ; Carter & Hauler, 2000 ; Collins et al., 2012 ; Drew et al., 2007 ; Hardy, 2010 ; Hernandez et al., 2010 ; Hixson et al., 2016 ; Jirsa et al., 2015 ; Lin & Luo, 2011 ; Shapawi et al., 2007 ; Yigit et al., 2006). Various protein-rich plant-derived ingredients such as soybean meal (SBM) and lupin meal (LM) have, due to their steady supply and favourable nutritional profiles, been concomitantly incorporated in the diets of farmed fish. Substitution of considerable amounts of FM with plant-derived proteins has been achieved in the salmon industry. Despite this advancement, complete replacement of FM in aquaculture feeds remains difficult. For carnivorous marine finfish, plant-derived proteins possess nutritional drawbacks including inappropriate amino acids (AA) balance (Naylor et al., 2000), the presence of certain anti-nutritional factors (ANF) (Gatlin et al., 2007) and poor protein digestibility (Krogdahl et al., 2010), thus forcing commercial feed production to heavily rely on marine ingredients containing high FM content.

A large number of studies has been conducted to investigate the influences of replacing FM with plant-based ingredients in aquaculture feeds. Many of these studies reported an impressive reduction in the levels of FM included in compounded feed among major groups of aquaculture species. However, the most common finding highlights that while partial substitution of FM results in fair growth, high or complete substitution appears to be unsuccessful due to issues concerning the inferior characteristics of PP to FM. In light of the debate on the sustainability of FM and rising FM prices, the inclusion of alternative ingredients in compounded feeds will improve both the ecological and economical sustainability of the carnivorous marine finfish aquaculture industry. Improvement of the nutritional quality of plant-derived protein products is, therefore, crucial for producing aquaculture feed that meets the nutritional needs of cultured fish.

A series of laboratory trials were employed in this study to evaluate the effects of selenium (Se) supplementation and the high replacement of FM with PP-based diets for two different carnivorous marine finfish species, namely yellowtail kingfish (*Seriola lalandi*) and barramundi (*Lates calcarifer*). This study focused on the application of nutritional improvement strategies using selenium (Se) as a feed supplement and or refinement of PP ingredients to improve their inclusion level in the diets of these fish and thus, to maximise aquaculture production.

9.1.1. Supplementation of organic Se (OS) in yellowtail kingfish fed FM and PP-based diets

For aquaculture nutritionists, the characterisation of the optimal quantity of specific nutrients and their interaction with environmental temperature is important for optimising the nutrient utilisation required for normal growth. Se is an essential micronutrient required by fish for normal growth and physiological function. In the first experiment, the effect of a Se-supplemented FM-based diet on the growth and health performance of juvenile yellowtail kingfish at two different temperatures was studied. The results demonstrated that both water temperature and Se level interactively affected the growth performance of yellowtail kingfish, with the overall final weight (FW) and specific growth rate (SGR) higher at 21ºC (128.7 g and 2.2% body weight day^{-1}) than at 26ºC (101.7 g and 1.51 body weight day^{-1}). For fish fed a Se-deficient diet, low SGR was found in both experimental temperatures. The

experiment suggested the optimum dietary Se concentration for yellowtail kingfish to be ~5.46 mg Se kg^{-1} (Chapter 3). This finding is in agreement with earlier studies on Se nutrition for the same species (Le et al., 2014a ; Le & Fotedar, 2013b).

Differences in SGR between Chapter 3 and Chapter 4 were due to the initial size of the fish used in both experiments, being 67 g and 5 g, respectively. OS supplementation in FM and PP diets for yellowtail kingfish were accomplished without affecting SGR (Figure 9.1). The positive effect of OS supplementation on the SGR of yellowtail kingfish was consistent with the results reported for other marine finfish fed FM-based diets (Zhu et al., 2012 ; Arshad et al., 2011 ; Liu et al., 2010 ; Lin & Shiau, 2005). Improved SGR of yellowtail kingfish fed diets containing PP ingredients supplemented with OS may have been due to an increase in antioxidant enzymatic activity, as indicated in the case of rainbow trout (*Oncorhynchus mykiss*) (Fontagne-Dicharry et al., 2015).

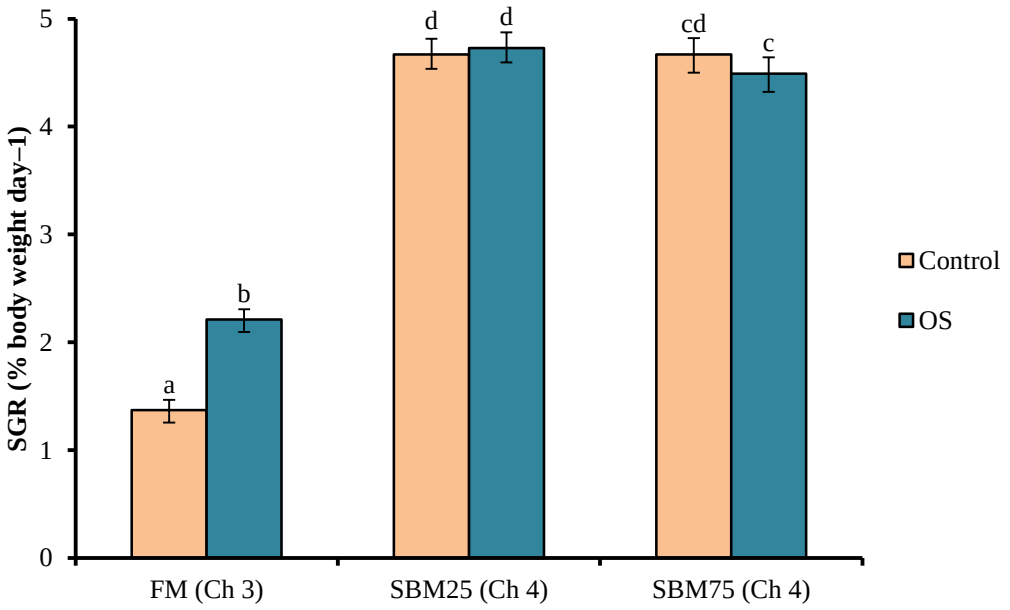

Figure 9.1. SGR of yellowtail kingfish fed FM and SBM diets supplemented with OS. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

Se, via selenoproteins, has been shown to play a fundamental role in activating the antioxidant defence system as it forms selenocysteine, which is the active site of the antioxidant enzymes glutathione peroxidase (GPx). OS-fed yellowtail kingfish attained higher GPx activity, except when high SBM was included in their diets

(Figure 9.2). Indeed, Fontagne-Dicharry et al. (2015) observed that GPx activity in rainbow trout (*O. mykiss*) did not significantly change when FM was entirely replaced with plant meals. Lower GPx activity among fish fed high SBM diets (as found in the present study) may be linked to antinutritional phytate inhibiting the normal absorption of Se (Chapter 4).

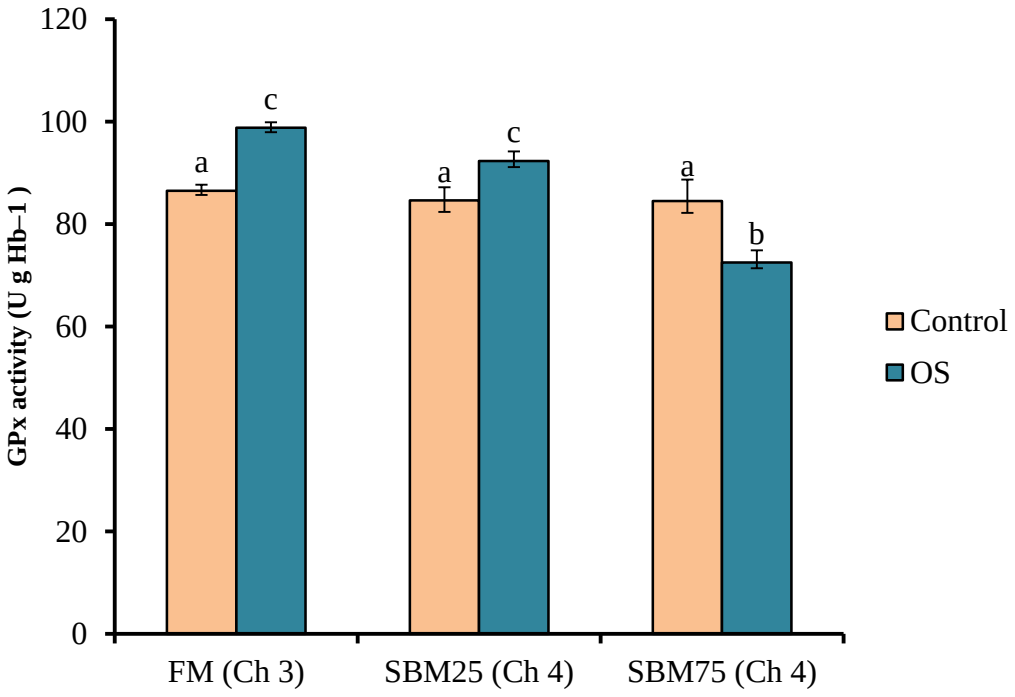

Figure 9.2. GPx activity of yellowtail kingfish fed FM and SBM diets supplemented with OS. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

The present study evidently indicates that yellowtail kingfish have a requirement for Se that cannot be fulfilled by Se-free diets. Results presented in Chapter 3 and Chapter 4 clearly demonstrate both the role of Se and advancements in the replacement of FM with lower-cost plant feedstuffs such as SBM. Dietary formulation identified in this study may, therefore, be important and can potentially serve as the basis for the further development of PP-based diets for yellowtail kingfish and other carnivorous marine finfish species.

9.1.1. Supplementation of organic Se (OS) in barramundi fed PP-based diets

In terms of growth, juvenile barramundi fed high PP diets supplemented with OS outperformed the those fed high PP diets lacking OS supplementation (Chapter 5 and

Chapter 6). PP ingredients such as SBM and LM commonly contain substantial amounts of ANFs. Among these, phytate has been known to bind minerals, reducing their absorption and bioavailability to fish (Kumar et al., 2012). Reduction in mineral utilisation and growth induced by phytate-containing PP-based diets have been reported for striped bass (*Morone saxatilis*) (Papatryphon et al., 1999) and Japanese flounder (*Paralichthys olivaceus*) (Laining et al., 2010). Therefore, in the present study, an increased level of Se in SBM- and LM-based diets supplemented with OS may have induced reasonably better performance compared to diets without OS supplementation. Recently, Se-yeast supplementation has been observed to increase the inclusion level of SBM in the diets of golden pompano (*Trachinotus ovatus*) (Wang et al., 2016a). The significance of Se for the stimulation of growth has been established for various finfish species including grouper (*Epinephelus malabaricus*) (Lin & Shiau, 2005), cobia (*Rachycentron canadum*) (Liu et al., 2010), yellowtail kingfish (*S. lalandi*) (Le et al., 2014a ; Le & Fotedar, 2013b) and Nile tilapia *Oreochromis niloticus* (Lee et al., 2016).

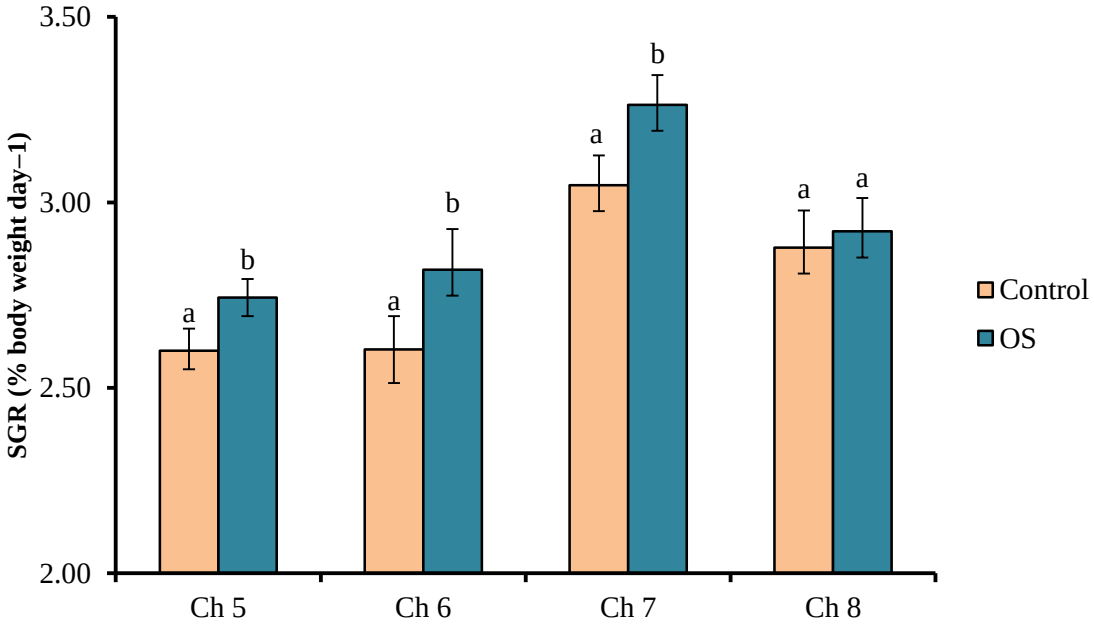

Figure 9.3. SGR of barramundi fed high PP diets supplemented with OS. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

To improve the nutritional quality and thus the inclusion level of PP ingredients in the diets of juvenile barramundi, test PP ingredients were fermented. The present findings clearly demonstrated that fermentation successfully improved the SGR, as

the fish fed fermented PP diets performed better than those fed non-fermented PP diets (Figure 9.3). Similar findings were reported for barramundi (*L. calcarifer*) fed diets containing 60% fermented lupin kernels (Van Vo et al., 2015). Fermented SBM was reported as proper protein sources in the diets of black sea bream (*Acanthopagrus schlegelii*) (Zhou et al., 2011 ; Azarm & Lee, 2014). In addition, fermented SBM is the most promising ingredient in FM-free diets for rainbow trout (*O. mykiss*) (Yamamoto et al., 2012 ; Murashita et al., 2013). However, supplementation of OS to a fermented plant-based diet has been shown to induce better growth, particularly in the case of SBM (Figure 9.3). These results explicate the accumulated benefits of both the bioprocessing of PP ingredients and the supplementation of OS.

The present study shows that AA content can be significantly increased by 17% in SBM and 30% in LM by solid-state fermentation (Chapter 7 and Chapter 8). Fermented SBM ingredients, when fed to juvenile barramundi (*L. calcarifer*), gave better SGR than feeding non-fermented SBM ingredients at similar dietary inclusion levels. In comparison, despite the higher AA content of fermented LM, the growth of fish fed fermented LM-based diets was not significantly different from that of fish fed non-fermented LM diets. It is also interesting to note that, although AA content was improved in fermented LM when compared to fermented SBM, the SGR of fish fed fermented SBM diets was higher than those fed fermented LM diets. This may be associated with the presence and level of ANF in these two PP sources, as observed in several finfish species (Van Vo et al., 2015 ; Shiu et al., 2015a ; Nguyen et al., 2015 ; Zhou et al., 2011 ; Yamamoto et al., 2010 ; Refstie et al., 2005).

It is well known that phytic acid, or phytate, has the ability to bind with macro- and micro-minerals (Kumar et al., 2012 ; Zhu et al., 2015 ; von Danwitz et al., 2016). Fermentation with *Saccharomyces cerevisiae* is a recommended practice for degrading the level of phytate in PP feedstuffs through the production of microbial phytases, which remove phosphate groups (Tudor et al., 2013) and thereby allowing an increase in mineral bioavailability. In the present study, fermentation reduced the phytate content of SBM and LM by roughly 68% and 70%, respectively, as shown in Figure 9.4. Development of fermentation techniques to produce phytate-free PP sources for aquaculture feed, therefore, deserves further investigation.

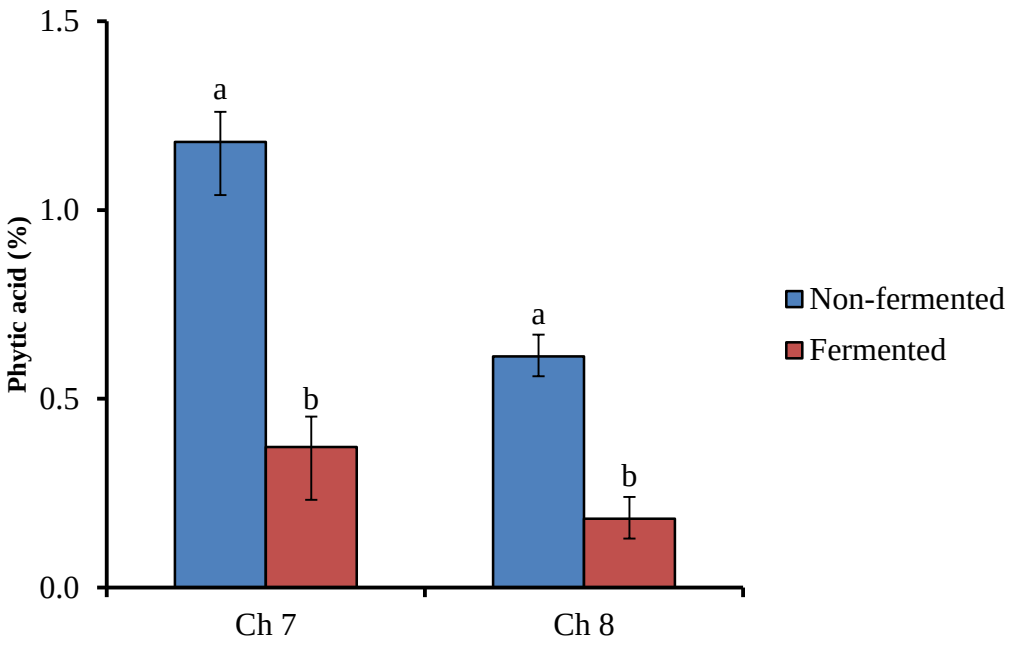

Figure 9.4. Phytic acid content of plant-based ingredients (SBM and LM) used in the experimental diets. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

High substitution of FM with PP ingredients may lead to a Se deficiency, since Se content is higher in FM than in PP materials (Hertrampf & Piedad-Pascual, 2000). As an essential constituent of GPx, the amount of Se intake affects antioxidant capacity (Zhu et al., 2012 ; Wang et al., 2012), and Se deficiency swiftly decreases growth and GPx activity. For example, hybrid striped bass (*Morone saxatilis* × *M. chrysops)* juveniles fed SBM or casein-based diets lacking Se supplementation caused retarded growth and reduced GPx activity, compared to those fed FM-based diets (Cotter, 2006). In rainbow trout (*O. mykiss*), SBM diets without Se supplementation was required to maintain optimum GPx activity, although the level of Se in Se-unsupplemented diets was adequate in terms of growth performance (Fontagne-Dicharry et al., 2015). The current research suggests that, in an attempt to increase the inclusion of PP sources in diets, the dietary Se level required for maintaining the maximal growth and GPx activity of juvenile barramundi is between 3.5 and 4.5 mg Se kg^{-1}. Fish fed diets below and above this level showed growth depression and histopathological changes.

Unlike previous studies with rainbow trout (Bell et al., 1986 ; Fontagne-Dicharry et al., 2015), this research demonstrated a substantial decrease in growth attained by Se-deficient fish. This was likely caused by the levels of ANF (e.g. phytic acid)

contained in the PP-based diets. It may, on the other hand, signify a higher requirement for Se by barramundi fed high PP-based diets. This notion is supported by the fact that GPx activity in control barramundi was nearly 10 times that in control rainbow trout. As with yellowtail kingfish, a higher Se requirement in barramundi may be sufficiently fulfilled by an FM-based diet, as commonly comparable to commercial diets. However, replacement of FM protein with high amounts of PP sources will affect Se content, for which dietary re-formulation or evaluation is required.

Se is an essential constituent of major biological functions, including thyroid hormone metabolism, antioxidant defence systems, and immune responses (Brown & Arthur, 2001 ; McKenzie et al., 2002). Se influences immune functions by regulating the activity of GPx (Felton et al., 1990). In the present study, a significant increase in erythrocyte GPx activity was noticed as a result of OS supplementation in diets (Figure 9.5). Taking this into account, the lower inhibition of growth, physiological and histopathological alterations in juvenile barramundi can be portrayed as a result of its protection by the enzymatic antioxidant capacity of GPx. Similar GPx responses to dietary Se concentration has been reported for grouper (*E. malabaricus*) (Lin & Shiau, 2005), rainbow trout (*O. mykiss*) (Kucukbay et al., 2009), cobia (*R. canadum*) (Liu et al., 2010), and whiteleg shrimp (*Penaeus vannamei*) (Wang et al., 2006a). Dhur et al. (1990) observed that a decline in GPx activity induced by Se malnutrition can favour the formation of increased cellular obliteration by H_2O_2 and other toxic radicals. The results of the current study clearly suggest that Se-dependent enzyme activities in barramundi are maintained based on dietary Se intake and that supplemental OS is necessary for meeting juvenile barramundi requirements when fed high PP-based diets.

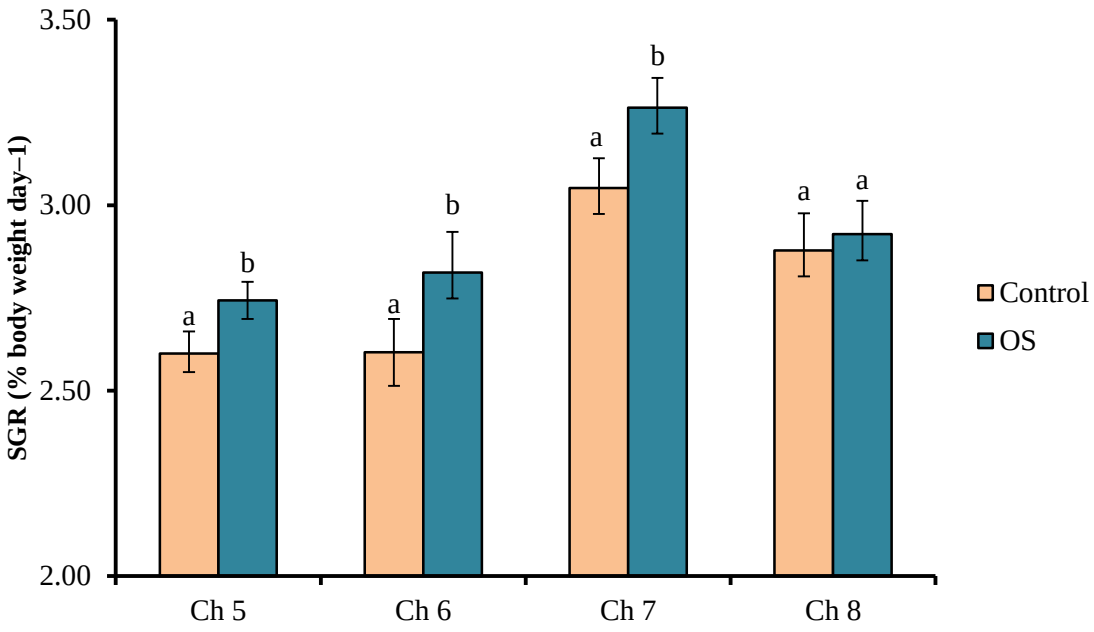

Figure 9.5. GPx activity of barramundi fed high PP diets supplemented with OS. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

Furthermore, histopathological changes were observed under light micrographs for muscle tissue, and fibre structures being distorted, disconnected and ruptured in both control and Se-deficient fish (Chapter 5 and Chapter 6). The levels of muscle damage achieved were identical to those reported for Se-deficient yellowtail kingfish (Le et al., 2014b ; Ilham & Fotedar, 2016).

In the present study, both plasma GPx activity and muscle Se level were significantly increased in OS-supplemented groups (Figure 9.5 and Figure 9.6). In line with this, most authors agree that Se status is correlated to GPx activity in fish (Abdel-Tawwab et al., 2007 ; Bell et al., 1986 ; Dörr et al., 2008 ; Ilham et al., 2016a ; Ilham et al., 2016c ; Jaramillo Jr et al., 2009 ; Kucukbay et al., 2009 ; Le & Fotedar, 2013b ; Lin & Shiau, 2005 ; Penglase et al., 2010 ; Wang et al., 2016a ; Zhu et al., 2012). These results may indicate that both muscle Se concentration and plasma GPx activity can be applied as biomarkers for expressing Se status in marine carnivorous finfish species, particularly when they are fed diets containing high amounts of PP ingredients.

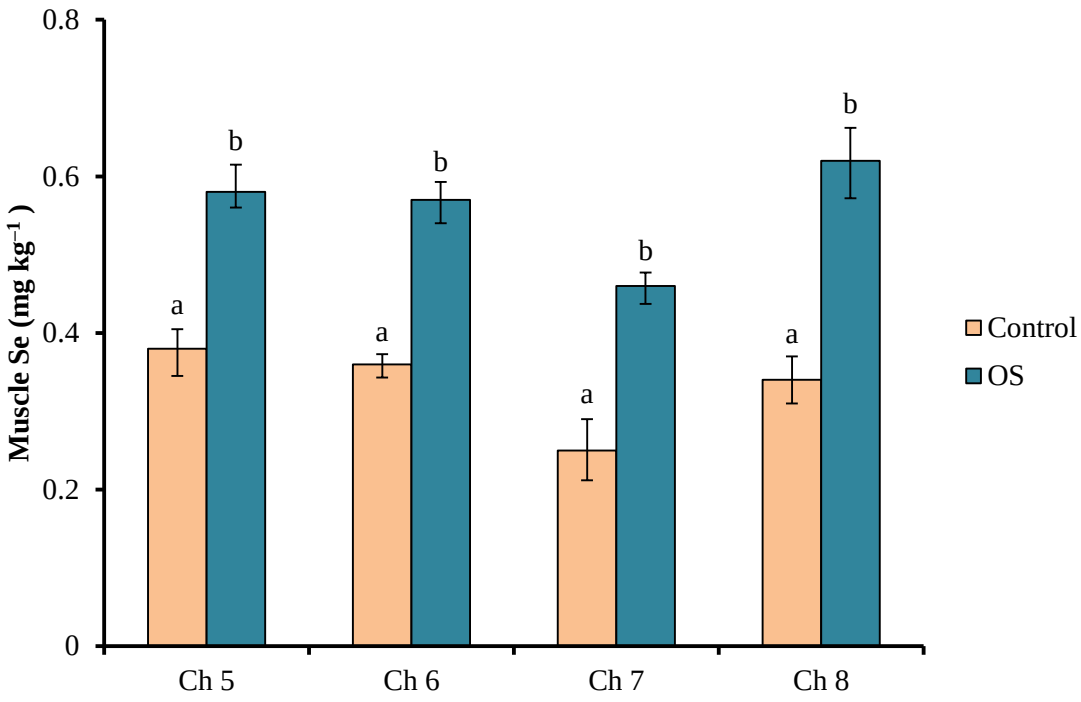

Figure 9.6. Muscle Se concentration of barramundi fed high PP diets supplemented with OS. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

The blood chemistry of barramundi was previously assayed (Glencross et al., 2016, 2011). The measured blood biochemistry parameters of 73 g barramundi fed with FM diets including alanine aminotransferase (ALT) and creatinine kinase (CK) were 10 U L^{-1} and 3293 U L^{-1}, respectively (Glencross et al., 2011). The present study reveals that both fermentation and OS supplementation are able to maintain the level of plasma ALT activity of fish fed SBM-based diets at roughly 12 U L^{-1}, which is similar to the level of FM-fed fish (Chapter 7). In contrast, increased plasma ALT activity was observed in the blood of fish fed a fermented diet lacking OS supplementation. This may be linked to the presence of phytic acid, as explained above (see Figure 9.4). When phytic acid was greatly reduced via the fermentation process, the ALT activity was similar to that of fish fed the control FM diet, as demonstrated with LM in the present study (Figure 9.7).

Increased ALT activity in a blood biochemical profile is indicative of liver damage (López et al., 2015). Indeed, increased ALT activity, as presented in Chapter 8, coincides with the findings reported by Ilham et al. (2016a), in which lipid droplet congregation in hepatocytes was found. Elevated levels in the activities of ALT is due to the response of fish to nutritional stressors, as shown in starry flounder

(*Platichthys stellatus*) (Song et al., 2014), although this was not the case for gilthead sea bream (*Sparus aurata*) and beluga (*Huso huso*) (Benedito-Palos et al., 2016 ; Soltanzadeh et al., 2015). However, it is believed that the level of ALT enzymes in fish may vary according to species, age, nutritional status, and environmental conditions (Shi et al., 2006 ; Chen et al., 2003).

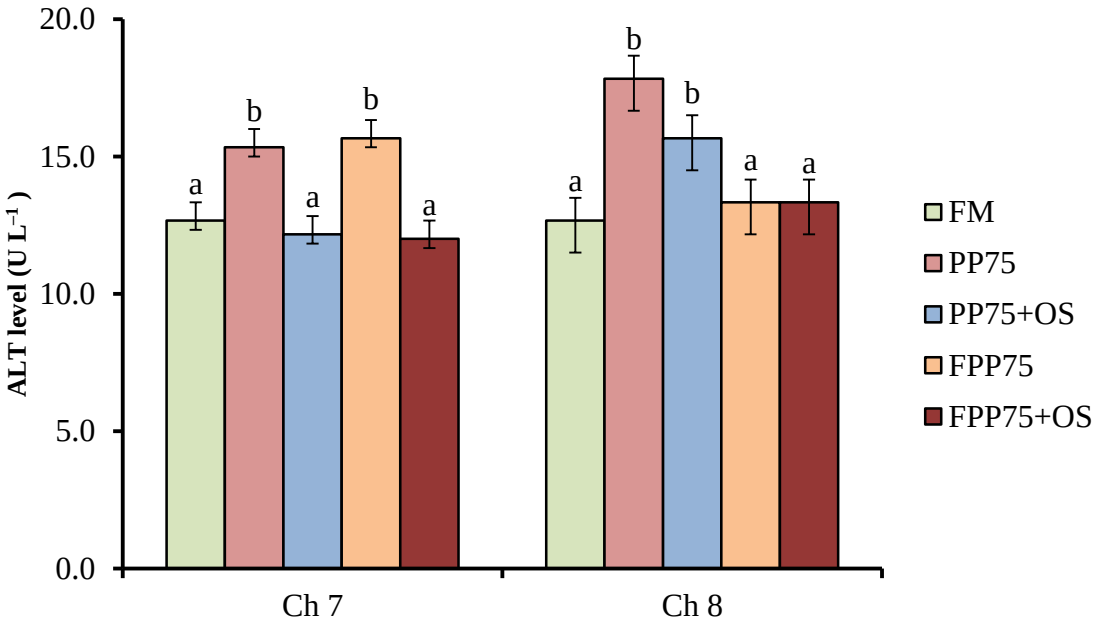

Figure 9.7. Alanine aminotransferase concentration of barramundi fed high PP diets supplemented with OS. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

Another effect of dietary fermented PP and OS supplementation is a decrease in CK, an important plasma enzyme that is commonly used as a biochemical indicator of muscle health. An increase in CK value is correlated with progressive muscle damage (Chen et al., 2003). In the present study, dietary fermented PP-based diets supplemented with OS promoted an appropriate CK level, similar to that of FM-fed fish (Figure 9.6). With SBM, the CK level decreased significantly when either OS supplementation or fermentation was applied; however, the proper level was achieved by combined OS supplementation and fermentation (Chapter 7). To compare, when LM was employed to replace FM, OS supplementation or the fermentation of LM ingredients was enough to maintain the CK level required for normal blood physiology (Chapter 8). Se-induced myopathy in juvenile barramundi fed with PP-based diets as previously reported by Ilham et al. (2016a,b) may have been the result of CK activity. The findings of the current study indicate that diet

formulation strategies play an important role in adjusting functional changes in blood cells and as a result, biochemical status of the cultured fish. Therefore, dietary fermented PP supplemented with OS serves as a promising FM replacer in carnivorous marine finfish.

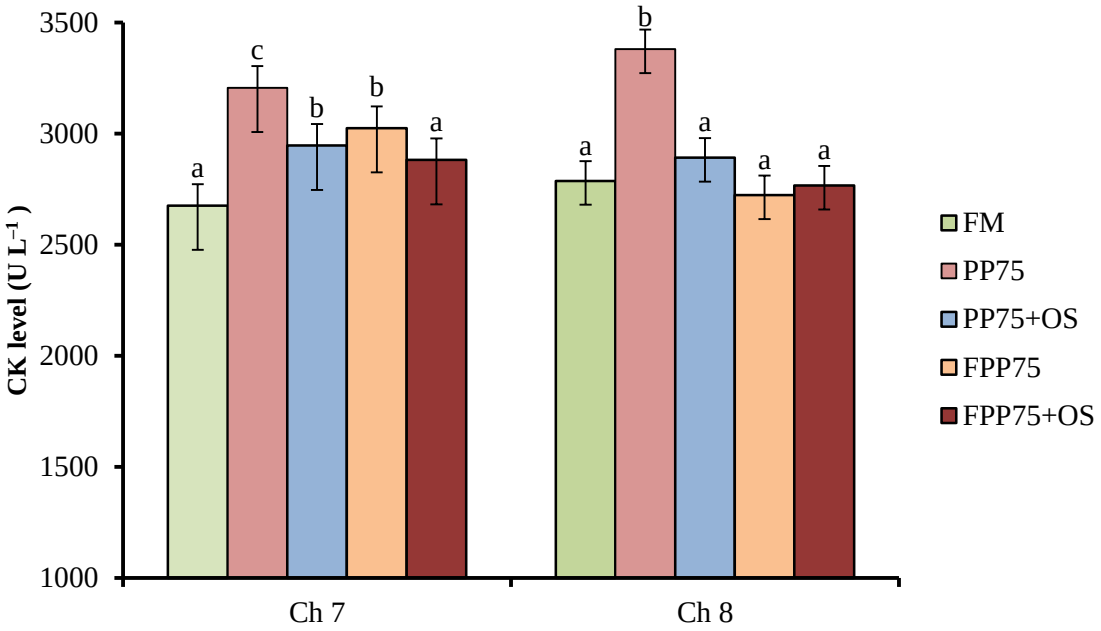

Figure 9.8. Creatinine kinase concentration of barramundi fed high PP diets supplemented with OS. The different letters (a, b) above the bars in the same chapter data series indicate significantly different means ($P < 0.05$).

9.2. Conclusions

Based on the results of this research, the following conclusions are drawn:

1. Dietary OS supplementation significantly improved the SGR of yellowtail kingfish and barramundi.
2. Optimum dietary Sel-Plex® inclusion level is 2 g kg^{-1} in the diet to maintain the growth and enzymatic GPx activity of yellowtail kingfish and barramundi.
3. With the supplementation of OS, 25% FM protein can be replaced with PP ingredient in yellowtail kingfish; however, 75% FM protein replacement with OS-supplemented PP sources is plausible for barramundi.
4. There is a strong correlation between dietary Se intake and muscle Se concentration in marine finfish such as yellowtail kingfish and barramundi.
5. Under Se-deficient conditions, there will be a greater incidence of histopathological alterations that may expose fish to serious diseases.

6. GPx activity was modified by dietary Se concentration and the enhancement of GPx activity in fish fed with dietary OS, which may have prevented muscle lesions and lipid congregation in hepatocytes.
7. Supplementation of 2 mg OS kg^{-1} in PP-containing diets is necessary to counteract the negative effect of antinutritional phytate, and thereby increasing Se bioavailability for fish.
8. Supplementation of 2–2.5 mg OS kg^{-1} in fermented PP-containing diets is recommended to promote proper blood haematology and the biochemical characteristics of juvenile barramundi.
9. Fermented SBM and LM with *S. cerevisiae* simultaneously supplemented with 2 mg OS kg^{-1} promotes sustainable diets for carnivorous marine finfish.

9.3. Recommendations

Based on the findings of this research, the following recommendations are made:

1. It is necessary to have sufficient knowledge of mineral utilisation at relevant environmental conditions to improve dietary composition and feeding conditions, thereby enhancing fish performance during the culture period. Therefore, more environmental variables need to be investigated to determine any interactions with dietary supplementation of minerals.
2. Further work is needed to evaluate whether the inclusion of more refined plant-derived proteins can improve the substitution level of PP sources in yellowtail kingfish diets.
3. Future research should consider the lipid and fatty acid profiles of muscle tissue, which may be directly related the Se-dependent GPx for the regulation of cell protection from oxidative damage.
4. In Se nutritional-related studies, the requirements and toxicity for Se are narrow; thus, care must be exercised when selecting supplementation level for diet enhancement purposes.
5. The most suitable technique for the complete removal of phytate and other ANFs deserves further investigation.
6. Future studies should include the secretion of digestive enzymes in the supplementation of fermented and non-fermented plant-derived proteins.

7. Interaction of temperature and pH in Se-supplemented diet in fermented and non-fermented plant-based ingredients is also required to take up this study at the commercial level.
8. Culture system and culture duration involved in the study was at the pilot level and, therefore, further studies in the commercial systems will definitely help to improve growth performance of PP-fed fish.

REFERENCES

ABARES (2014) Australian Fisheries and Aquaculture Statistics 2013. Department of Agriculture Australia, Canberra.

Abbink W, Blanco Garcia A, Roques JAC, Partridge GJ, Kloet K, Schneider O (2012) The effect of temperature and pH on the growth and physiological response of juvenile yellowtail kingfish *Seriola lalandi* in recirculating aquaculture systems. *Aquaculture,* **330–333**, 130-135.

Abdel-Tawwab M, Mousa MAA, Abbass FE (2007) Growth performance and physiological response of African catfish, *Clarias gariepinus* (B.) fed organic selenium prior to the exposure to environmental copper toxicity. *Aquaculture,* **272**, 335-345.

Abdul Kader M, Koshio S, Ishikawa M, Yokoyama S, Bulbul M, Honda Y, Mamauag R, Laining A (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. *Fish. Sci.,* **77**, 119-128.

Adams SM, Brown AM, Goede RW (1993) A quantitative health assessment index for rapid rvaluation of fish condition in the field. *Trans. Am. Fis. Soc.,* **122**, 63-73.

Adelizi, Rosati, Warner, Wu, Muench, White, Brown (1998) Evaluation of fish-meal free diets for rainbow trout, *Oncorhynchus mykiss*. *Aquac. Nutr.,* **4**, 255-262.

Akiyama T, Mori K, Murai T (1986) Effects of temperature on the incidence of scoliosis and cataracts in chum salmon fry caused by tryptophan deficiency. *Bull. Jap. Soc. Sci. Fish.,* **52**, 2039.

Al-Dohail MA, Hashim R, Aliyu-Paiko M (2009) Effects of the probiotic, Lactobacillus acidophilus, on the growth performance, haematology parameters and immunoglobulin concentration in African Catfish (*Clarias gariepinus*, Burchell 1822) fingerling. *Aquac. Res.*, **40**, 1642-1652.

Alexis MN (1990) Comparative evaluation of soybean meal and carob seed germ meal as dietary ingredients for rainbow trout fingerlings. *Aquat. Living Resour.,* **3**, 235-241.

Allan GL, Booth MA (2004) Effects of extrusion processing on digestibility of peas, lupins, canola meal and soybean meal in silver perch *Bidyanus bidyanus* (Mitchell) diets. *Aquac. Res.,* **35**, 981-991.

Allsopp M, Johnston P, Santillo D (2008) Challenging the aquaculture industry on sustainability: technical overview. *Greenpeace Research Laboratories Technical Note,* **1**, 59.

Anderson IG, Schaumuller LF, Kramer HL (1996) A preliminary study on the hematology of freshwater-reared sea bass/barramundi, *Lates calcarifer*. *Asian Fish. Sci.,* **9**, 101-107.

Anderson RL, Wolf WJ (1995) Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. *J. Nutr.,* **125**, 581S-588S.

Andreeva AM (2012) *Structural and Functional Organization of Fish Blood Proteins*, Nova Science Publishers Inc, New York.

Angilletta MJJ, Dunham AE (2003) The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general. *Am. Nat.,* **162**, 332-342.

AOAC (1990) *Official methods of analysis of the Association of Official Analytical Chemists*, The Association of Official Analytical Chemists, Arlington.

Aoki H, Furuichi M, Viyakarn V, Yamagata Y, Watanabe T (1998) Feed protein ingredients for red sea bream. *Suisanzoshoku*, **46**, 121-127.

Apines-Amar MJS, Satoh S, Caipang CMA, Kiron V, Watanabe T, Aoki T (2004) Amino acid-chelate: a better source of Zn, Mn and Cu for rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **240**, 345-358.

Ardiansyah, Fotedar R (2016) Water quality, growth and stress responses of juvenile barramundi *(Lates calcarifer* Bloch), reared at four different densities in integrated recirculating aquaculture systems. *Aquaculture*, **458**, 113-120.

Arndt RE, Hardy RW, Sugiura SH, Dong FM (1999) Effects of heat treatment and substitution level on palatability and nutritional value of soy defatted flour in feeds for Coho Salmon, *Oncorhynchus kisutch*. *Aquaculture,* **180**, 129-145.

Arshad U, Takami GA, Sadeghi M, Bai S, Pourali HR, Lee S (2011) Influence of dietary l-selenomethionine exposure on growth and survival of juvenile *Huso huso*. *J. Appl. Ichthyol.,* **27**, 761-765.

Arthur JR, McKenzie RC, Beckett GJ (2003) Selenium in the immune system. *J. Nutr.,* **133**, 1457S-1459S.

Asbridge DD (2015) Soybeans vs. Other Vegetable Oils as a Source of Edible Oil Products. In: *Practical Handbook of Soybean Processing and Utilization* (ed by Erickson DR). AOCS Press and the United Soybean Board., USA, pp. 130.

Ashida T, Okimasu E (2005) Immunostimulatory effects of fermented vegetable product on the non-specific immunity of Japanese flounder *Paralichthys olivaceus*. *Fish. Sci.*, **71**, 257-262.

Ashida T, Takel Y, Takagaki M, Matsuura Y, Okimasu E (2006) The dietary effects of a fermented vegetable product on glutathione peroxidase activity and lipid peroxidation of Japanese flounder *Paralichthys olivaceus*. *Fish. Sci.,* **72**, 179-184.

Ashmead HD (1992) *The Roles of Amino Acids Chelates in Animal Nutrition*, Noyes Publications, Park Ridge, New Jersey.

Ashouri S, Keyvanshokooh S, Salati AP, Johari SA, Pasha-Zanoosi H (2015) Effects of different levels of dietary selenium nanoparticles on growth performance, muscle composition, blood biochemical profiles and antioxidant status of common carp (*Cyprinus carpio*). *Aquaculture,* **446**, 25-29.

Atencio L, Moreno I, Jos Á, Prieto AI, Moyano R, Blanco A, Cameán AM (2009) Effects of dietary selenium on the oxidative stress and pathological changes in tilapia (*Oreochromis niloticus*) exposed to a microcystin-producing cyanobacterial water bloom. *Toxicon,* **53**, 269-282.

Austreng E (1978) Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. *Aquaculture*, **13**, 265-272.

Azarm HM, Lee S-M (2014) Effects of partial substitution of dietary fish meal by fermented soybean meal on growth performance, amino acid and biochemical parameters of juvenile black sea bream *Acanthopagrus schlegeli*. *Aquac. Res.*, **45**, 994-1003.

Azaza MS, Wassim K, Mensi F, Abdelmouleh A, Brini B, Kraïem MM (2009) Evaluation of faba beans (*Vicia faba* L. var. minuta) as a replacement for soybean meal in practical diets of juvenile Nile tilapia *Oreochromis niloticus*. *Aquaculture*, **287**, 174-179.

Bandyopadhyay P, Das Mohapatra P (2009) Effect of a probiotic bacterium Bacillus circulans PB7 in the formulated diets: on growth, nutritional quality and immunity of *Catla catla* (Ham.). *Fish Physiol. Biochem.,* **35**, 467-478.

Bangoula D, Parent J, Vellas F (1993) Nutritive value of white lupin *Lupinus albus* var Lutop fed to rainbow trout (*Oncorhynchus mykiss)*. Effects of extrusion cooking. *Reprod. Nutr. Dev.,* **33**, 325-334.

Barciela J, Herrero C, García-Martín S, Peña RM (2008) A brief study of the role of Selenium as antioxidant. *Elec. J. Env. Agricult. Food Chem.,* **7**, 3151-3155.

Barkholt V, Jensen AL (1989) Amino acid analysis: determination of cysteine plus half-cystine in proteins after hydrochloric acid hydrolysis with a disulfide compound as additive. *Anal. Biochem.,* **177**, 318-322.

Barros MM, Lim C, Klesius PH (2002) Effect of soybean meal replacement by cottonseed meal and iron supplementation on growth, immune response and resistance of channel catfish (*Ictalurus puctatus*) to *Edwardsiella ictaluri* challenge. *Aquaculture,* **207**, 263-279.

Barrows FT, Stone DAJ, Hardy RW (2007) The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **265**, 244-252.

Bartkiene E, Krungleviciute V, Juodeikiene G, Vidmantiene D, Maknickiene Z (2015) Solid state fermentation with lactic acid bacteria to improve the nutritional quality of lupin and soya bean. *J. Sci. Food Agric.,* **95**, 1336-1342.

Barton BA, Iwama GK (1991) Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annu. Rev. Fish Dis.,* **1**, 3-26.

Baruah K, Pal AK, Sahu NP, Debnath D, Nourozitallab P, Sorgeloos P (2007) Microbial phytase supplementation in rohu, *Labeo rohita*, diets enhances growth performance and nutrient digestibility. *J. World Aquacult. Soc.,* **38**, 129-137.

Bautista-Teruel MN, Eusebio PS, Welsh TP (2003) Utilization of feed pea, *Pisum sativum*, meal as a protein source in practical diets for juvenile tiger shrimp, *Penaeus monodon*. *Aquaculture,* **225**, 121-131.

Belewu M, Sam R (2010) Solid state fermentation of Jatropha curcas kernel cake: proximate composition and antinutritional components. *J. Yeast Fungal Res.,* **1**, 44-46.

Bell JG, Cowey CB (1989) Digestibility and bioavailability of dietary selenium from fishmeal, selenite, selenomethionine and selenocystine in Atlantic salmon (*Salmo salar*). *Aquaculture,* **81**, 61-68.

Bell JG, Cowey CB, Adron JW, Pirie BJS (1987) Some effects of selenium deficiency on enzyme activities and indices of tissue peroxidation in Atlantic salmon parr (*Salmo salar*). *Aquaculture,* **65**, 43-54.

Bell JG, Cowey CB, Adron JW, Shanks AM (1985) Some effects of vitamin E and selenium deprivation on tissue enzyme levels and indices of tissue peroxidation in rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.,* **53**, 149-157.

Bell JG, Pirie BJS, Adron JW, Cowey CB (1986) Some effects of selenium deficiency on glutathione peroxidase (EC 1.11.1.9) activity and tissue pathology in rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.,* **55**, 305-311.

Bendiksen EÅ, Johnsen CA, Olsen HJ, Jobling M (2011) Sustainable aquafeeds: Progress towards reduced reliance upon marine ingredients in diets for farmed Atlantic salmon (*Salmo salar* L.). *Aquaculture,* **314**, 132-139.

Benedito-Palos L, Ballester-Lozano GF, Simó P, Karalazos V, Ortiz Á, Calduch-Giner J, Pérez-Sánchez J (2016) Lasting effects of butyrate and low FM/FO diets on growth performance, blood haematology/biochemistry and molecular growth-related markers in gilthead sea bream (*Sparus aurata*). *Aquaculture,* **454**, 8-18.

Bermudes M, Glencross B, Austen K, Hawkins W (2010) The effects of temperature and size on the growth, energy budget and waste outputs of barramundi (*Lates calcarifer*). *Aquaculture,* **306**, 160-166.

Biswas AK, Kaku H, Ji SC, Seoka M, Takii K (2007) Use of soybean meal and phytase for partial replacement of fish meal in the diet of red sea bream, *Pagrus major*. *Aquaculture,* **267**, 284-291.

Bjerkås E, Bjørnestad E, Breck O, Waagbø R (2001) Water temperature regimes affect cataract development in smolting Atlantic salmon, *Salmo salar* L. *J. Fish Dis.,* **24**, 281-291.

Björnsson B, Steinarsson A, Oddgeirsson M (2001) Optimal temperature for growth and feed conversion of immature cod (*Gadus morhua* L.). *ICES J. Mar. Sci.,* **58**, 29-38.

Blaxhall PC (1972) The haematological assessment of the health of freshwater fish. *J. Fish Biol.,* **4**, 593-604.

Bonaldo A, Parma L, Mandrioli L, Sirri R, Fontanillas R, Badiani A, Gatta PP (2011) Increasing dietary plant proteins affects growth performance and ammonia excretion but not digestibility and gut histology in turbot (*Psetta maxima*) juveniles. *Aquaculture,* **318**, 101-108.

Bonaldo A, Roem AJ, Pecchini A, Grilli E, Gatta PP (2006) Influence of dietary soybean meal levels on growth, feed utilization and gut histology of Egyptian sole (*Solea aegyptiaca*) juveniles. *Aquaculture,* **261**, 580-586.

Boonyaratpalin M, Suraneiranat P, Tunpibal T (1998) Replacement of fish meal with various types of soybean products in diets for the Asian seabass, *Lates calcarifer*. *Aquaculture,* **161**, 67-78.

Booth MA, Moses MD, Allan GL (2013) Utilisation of carbohydrate by yellowtail kingfish *Seriola lalandi*. *Aquaculture,* **376–379**, 151-161.

Borlongan IG, Eusebio PS, Welsh T (2003) Potential of feed pea (*Pisum sativum*) meal as a protein source in practical diets for milkfish (*Chanos chanos* Forsskal). *Aquaculture,* **225**, 89-98.

Borquez A, Serrano E, Dantagnan P, Carrasco J, Hernandez A (2011) Feeding high inclusion of whole grain white lupin (*Lupinus albus*) to rainbow trout (*Oncorhynchus mykiss*): effects on growth, nutrient digestibility, liver and intestine histology and muscle fatty acid composition. *Aquac. Res.*, **42**, 1067-1078.

Bórquez AS, Hernández AJ, Dantagnan P, Saez P, Serrano E (2011) Incorporation of whole lupin, *Lupinus albus*, seed meal in commercial extruded diets for rainbow trout, *Oncorhynchus mykiss*: effect on growth performance, nutrient digestibility, and muscle fatty acid composition. *J. World Aquacult. Soc.,* **42**, 209-221.

Bostock J, McAndrew B, Richards R, Jauncey K, Telfer T, Lorenzen K, Little D, Ross L, Handisyde N, Gatward I (2010) Aquaculture: global status and trends. *Philos. Trans. R. Soc. Lond. B Biol. Sci.,* **365**, 2897-2912.

Bowden TJ, Thompson KD, Morgan AL, Gratacap RML, Nikoskelainen S (2007) Seasonal variation and the immune response: A fish perspective. *Fish Shellfish Immunol.,* **22**, 695-706.

Bowyer JN, Booth MA, Qin JG, D'Antignana T, Thomson MJS, Stone DAJ (2014) Temperature and dissolved oxygen influence growth and digestive enzyme activities of yellowtail kingfish *Seriola lalandi* (Valenciennes, 1833). *Aquac. Res.,* **45**, 2010-2020.

Bowyer JN, Qin JG, Smullen RP, Adams LR, Thomson MJS, Stone DAJ (2013a) The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*) feeds at different water temperatures: 1. Solvent extracted soybean meal. *Aquaculture,* **384–387**, 35-45.

Bowyer JN, Qin JG, Smullen RP, Adams LR, Thomson MJS, Stone DAJ (2013b) The use of a soy product in juvenile yellowtail kingfish (*Seriola lalandi*) feeds at different water temperatures: 2. Soy protein concentrate. *Aquaculture,* **410–411**, 1-10.

Bowyer JN, Qin JG, Stone DAJ (2013c) Protein, lipid and energy requirements of cultured marine fish in cold, temperate and warm water. *Rev. Aquac.,* **5**, 10-32.

Brown KM, Arthur JR (2001) Selenium, selenoproteins and human health: A review. *Public Health Nutr.,* **4**, 593-599.

Brown PB, Williams CD, Robinson EH, Akiyama DM, Lawrence AL (1986) Evaluation of methods for determining in vivo digestion coefficients for adult red swamp crayfish *Procambarus clarkii*. *J. World Aquacult. Soc.*, **17**, 19-24.

Buentello A, Jirsa D, Barrows FT, Drawbridge M (2015) Minimizing fishmeal use in juvenile California yellowtail, *Seriola lalandi*, diets using non-GM soybeans selectively bred for aquafeeds. *Aquaculture,* **435**, 403-411.

Bulbul M, Koshio S, Ishikawa M, Yokoyama S, Kader MA (2013) Performance of kuruma shrimp, *Marsupenaeus japonicus* fed diets replacing fishmeal with a combination of plant protein meals. *Aquaculture,* **372-375**, 45-51.

Burel C, Boujard T, Corraze G, Kaushik SJ, Boeuf G, Mol KA, Van Der Geyten S, Kühn ER (1998) Incorporation of high levels of extruded lupin in diets for rainbow trout (*Oncorhynchus mykiss*): nutritional value and effect on thyroid status. *Aquaculture,* **163**, 325-345.

Burk RF, Hill KE (1993) Regulation of selenoproteins. *Annu. Rev. Nutr.,* **13**, 65-81.

Burk RF, Hill KE, Awad JA, Morrow JD, Lyons PR (1995) Liver and kidney necrosis in selenium-deficient rats depleted of glutathione. *Lab. Invest.,* **72**, 723-730.

Caputo L, Visconti A, De Angelis M (2015) Selection and use of a *Saccharomyces cerevisae* strain to reduce phytate content of wholemeal flour during bread-

making or under simulated gastrointestinal conditions. *LWT - Food Sci. Tech.,* **63**, 400-407.

Carter CG, Hauler RC (2000) Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture,* **185**, 299-311.

Carton AG, Vaughan MR (2010) Behavioural and anatomical measures of visual acuity in first-feeding yellowtail kingfish (*Seriola lalandi*) larvae. *Environ. Biol. Fishes,* **89**, 3-10.

Castillo S, Gatlin DM (2015) Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A review. *Aquaculture,* **435**, 286-292.

Catacutan MR, Coloso RM (1995) Effect of dietary protein to energy ratios on growth, survival, and body composition of juvenile Asian seabass, *Lates calcarifer*. *Aquaculture,* **131**, 125-133.

Catacutan MR, Coloso RM (1997) Growth of juvenile Asian seabass, *Lates calcarifer*, fed varying carbohydrate and lipid levels. *Aquaculture,* **149**, 137-144.

Chariot P, Bignani O (2003) Skeletal muscle disorders associated with selenium deficiency in humans. *Muscle Nerve,* **27**, 662-668.

Chen BN, Qin JG, Kumar MS, Hutchinson W, Clarke S (2006) Ontogenetic development of the digestive system in yellowtail kingfish *Seriola lalandi* larvae. *Aquaculture,* **256**, 489-501.

Chen C-Y, Wooster GA, Getchell RG, Bowser PR, Timmons MB (2003) Blood chemistry of healthy, nephrocalcinosis-affected and ozone-treated tilapia in a recirculation system, with application of discriminant analysis. *Aquaculture,* **218**, 89-102.

Chen C, Berger J, Fletcher A, Lawes R, Robertson M (2015) Model-based evaluation of yield performance and agronomic options for lupins in the southern wheatbelt, Western Australia. In: *Proceedings of the 17th ASA Conference, 20 – 24 September*, Hobart, Australia.

Cheng ZJ, Hardy RW (2002) Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **212**, 361-372.

Chien YH, Chiu YH (2003) Replacement of soybean (*Glycine max* (L.) Merrill) meal by lupin (*Lupinus angustifolius*) seed meal in diet for juvenile tilapia (*Oreochromis niloticus* × *O. aureus*) reared indoors. *Aquac. Res.,* **34**, 1261-1268.

Chiu S-T, Hsieh S-L, Yeh S-P, Jian S-J, Cheng W, Liu C-H (2010) The increase of immunity and disease resistance of the giant freshwater prawn, *Macrobrachium rosenbergii* by feeding with selenium enriched-diet. *Fish Shellfish Immunol.,* **29**, 623-629.

Cho CY, Slinger SJ, Bayley HS (1982) Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comp. Biochem. Physiol.*, **73**, 25-41.

Chou RL, Her BY, Su MS, Hwang G, Wu YH, Chen HY (2004) Substituting fish meal with soybean meal in diets of juvenile cobia *Rachycentron canadum*. *Aquaculture*, **229**, 325-333.

Chu G-M, Ohmori H, Kawashima T, Funaba M, Matsui T (2009) Brewer's yeast efficiently degrades phytate phosphorus in a corn-soybean meal diet during soaking treatment. *Anim. Sci. J.*, **80**, 433-437.

Cleveland L, Little E, Buckler D, Wiedmeyer H (1993) Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). *Aquat. Toxicol.*, **27**, 265-280.

Collins SA, Desai AR, Mansfield GS, Hill JE, Van Kessel AG, Drew MD (2012) The effect of increasing inclusion rates of soybean, pea and canola meals and their protein concentrates on the growth of rainbow trout: Concepts in diet formulation and experimental design for ingredient evaluation. *Aquaculture*, **344–349**, 90-99.

Combs GF, Combs SB (1986) *Biochemical functions of selenium*, Academic Press, Toronto.

Connelly P (2011) Nutritional advantages and disadvantages of dietary phytates: Part 1. *J. Aust. Tradit-Med. Soc.*, **17**, 21-24.

Cotter P (2006) Dietary selenium in cultured hybrid striped bass. In: *Fisheries & Wildlife Sciences*. Virginia Polytechnic Institute and State University, Virginia, pp. 101.

Cotter PA, Craig SR, McLean E (2008) Hyperaccumulation of selenium in hybrid striped bass: a functional food for aquaculture? *Aquac. Nutr.*, **14**, 215-222.

Coughlan D, Velte J (1989) Dietary toxicity of selenium-contaminated red shiners to striped bass. *Trans. Am. Fis. Soc.*, **118**, 400-408.

Cuenco ML, Stickney RR, Grant WE (1985) Fish bioenergetics and growth in aquaculture ponds: II. Effects of interactions among, size, temperature, dissolved oxygen, unionized ammonia and food on growth of individual fish. *Ecol. Modell.*, **27**, 191-206.

Dainty JR, Fox TE (2005) Modeling in nutrition – The metabolism of selenium, copper, zinc and calcium using stable isotopes in humans. In: *Handbook of elemental speciation II – Species in the environment, food, medicine and occupational health* (ed. by Cornelis R, Caruso J, Crews H, Heumann K). John Wiley & Sons, Ltd, pp. 690-712.

Dang VT, Speck P, Benkendorff K (2012) Influence of elevated temperatures on the immune response of abalone, *Haliotis rubra*. *Fish Shellfish Immunol.*, **32**, 732-740.

Davies SJ, Gouveia A (2008) Enhancing the nutritional value of pea seed meals (*Pisum sativum*) by thermal treatment or specific isogenic selection with comparison to soybean meal for African catfish, *Clarias gariepinus*. *Aquaculture*, **283**, 116-122.

Davis KB, Griffin BR, Gray WL (2002) Effect of handling stress on susceptibility of channel catfish *Ictalurus punctatus* to *Ichthyophthirius multifiliis* and channel catfish virus infection. *Aquaculture*, **214**, 55-66.

Day O, Howell B (1997) Nutritional demands of farmed marine fish. *Nutr. Food Sci.*, **97**, 188-191.

de Almeida EA, Miyamoto S, Bainy ACD, de Medeiros MHG, Di Mascio P (2004) Protective effect of phospholipid hydroperoxide glutathione peroxidase (PHGPx) against lipid peroxidation in mussels *Perna perna* exposed to different metals. *Mar. Pollut. Bull.*, **49**, 386-392.

de la Higuera M, García-Gallego M, Sanz A, Cardenete G, Suárez MD, Moyano FJ (1988) Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout (*Salmo gairdneri*). *Aquaculture*, **71**, 37-50.

de Silva SS (1995) *Fish Nutrition in Aquaculture*, Chapman & Hall, London.

Delgado CL, Wada N, Rosegrant MW, Maijer S, Ahmed M (2003) Fish to 2020: Supply and demand in changing global market. In: *WorldFish Center Technical Report* International Food Policy Research Institute and WorldFish Center, Washington, pp. 225.

DFWA (2009) Finfish Aquaculture in Western Australia: Final ESD Management Report for Marine Finfish Aquaculture. In: *Fisheries Management Paper No. 233*, Perth, WA, Australia.

Dhur A, Galan P, Hercberg S (1990) Relationship between selenium, immunity and resistance against infection. *Comp. Biochem. Physiol. C: Pharmacol.*, **96**, 271-280.

Dias J, Gomes EF, Kaushik SJ (1997) Improvement of feed intake through supplementation with an attractant mix in European seabass fed plant-protein rich diets. *Aquat. Living Resour.*, **10**, 385-389.

Dietrich JP, Van Gaest AL, Strickland SA, Arkoosh MR (2014) The impact of temperature stress and pesticide exposure on mortality and disease susceptibility of endangered Pacific salmon. *Chemosphere*, **108**, 353-359.

Dörr AJM, Pacini N, Abete MC, Prearo M, Elia AC (2008) Effects of a selenium-enriched diet on antioxidant response in adult crayfish (*Procambarus clarkii*). *Chemosphere*, **73**, 1090-1095.

Drew MD, Borgeson TL, Thiessen DL (2007) A review of processing of feed ingredients to enhance diet digestibility in finfish. *Anim. Feed Sci. Tech.*, **138**, 118-136.

Dulski TR (1996) *A manual for the chemical analysis for metals,* American Society for Testing and Materials, West Conshohocken, PA.

Eckersall PD, Duthie S, Safi S, Moffatt D, Horadagoda NU, Doyle S, Parton R, Bennett D, Fitzpatrick JL (1999) An automated biochemical assay for haptoglobin: Prevention of interference from albumin. *Comp. Haem. Int.*, **9**, 117-124.

El-Asely AM, Abbass AA, Austin B (2014) Honey bee pollen improves growth, immunity and protection of Nile tilapia (*Oreochromis niloticus*) against infection with *Aeromonas hydrophila*. *Fish Shellfish Immunol.*, **40**, 500-506.

Elangovan A, Shim KF (2000) The influence of replacing fish meal partially in the diet with soybean meal on growth and body composition of juvenile tin foil barb (*Barbodes altus*). *Aquaculture*, **189**, 133-144.

Elia AC, Prearo M, Pacini N, Dörr AJM, Abete MC (2011) Effects of selenium diets on growth, accumulation and antioxidant response in juvenile carp. *Ecotoxicol. Environ. Saf.*, **74**, 166-173.

Espe M, Lemme A, Petri A, El-Mowafi A (2006) Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? *Aquaculture*, **255**, 255-262.

EU (2015) European Union register of feed additives pursuant to regulation (EC) No 1831/2003.

Fagbenro, Davies (2001) Use of soybean flour (dehulled, solvent-extracted soybean) as a fish meal substitute in practical diets for African catfish, *Clarias gariepinus* (Burchell 1822): growth, feed utilization and digestibility. *J. Appl. Ichthyol.*, **17**, 64-69.

FAO (2014) *The State of World Fisheries and Aquaculture*, Rome.

FAO (2016) *The State of World Fisheries and Aquaculture (SOFIA). Contributing to food security and nutrition for all*, FAO Fisheries and Aquaculture Department Rome.

Farhangi M, Carter CG (2001) Growth, physiological and immunological responses of rainbow trout (*Oncorhynchus mykiss*) to different dietary inclusion levels of dehulled lupin (*Lupinus angustifolius*). *Aquac. Res.*, **32**, 329-340.

Farhangi M, Carter CG (2007) Effect of enzyme supplementation to dehulled lupin-based diets on growth, feed efficiency, nutrient digestibility and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac. Res.*, **38**, 1274-1282.

Felton S, Ji W, Mathews S (1990) Selenium concentrations in coho salmon outmigrant smolts and returning adults: a comparison of wild versus hatchery-reared fish. *Dis. Aquat. Organ.*, **9**, 157-161.

Felton SP, Landolt ML, Grace R, Palmisano A (1996) Effects of selenium dietary enhancement on hatchery-reared coho salmon, *Oncorhynchus kisutch*

(Walbaum), when compared with wild coho: hepatic enzymes and seawater adaptation evaluated. *Aquac. Res.,* **27**, 135-142.

Feng J, Liu X, Xu ZR, Liu YY, Lu YP (2007) Effects of *Aspergillus oryzae* 3.042 fermented soybean meal on growth performance and plasma biochemical parameters in broilers. *Anim. Feed Sci. Tech.,* **134**, 235-242.

Fielder DS, Heasman MP (2011) Hatchery Manual for the Production of Australian Bass, Mulloway and Yellowtail Kingfish. Industry and Investment NSW.

Fontagne-Dicharry S, Godin S, Liu H, Antony Jesu Prabhu P, Bouyssiere B, Bueno M, Tacon P, Medale F, Kaushik SJ (2015) Influence of the forms and levels of dietary selenium on antioxidant status and oxidative stress-related parameters in rainbow trout (*Oncorhynchus mykiss*) fry. *Br. J. Nutr.,* **113**, 1876-1887.

Førde-Skjærvik O, Refstie S, Aslaksen MA, Skrede A (2006) Digestibility of diets containing different soybean meals in Atlantic cod (*Gadus morhua*); comparison of collection methods and mapping of digestibility in different sections of the gastrointestinal tract. *Aquaculture,* **261**, 241-258.

Francis G, Makkar HPS, Becker K (2001) Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture,* **199**, 197-227.

Fredlund K, Isaksson M, Rossander-Hulthen L, Almgren A, Sandberg AS (2006) Absorption of zinc and retention of calcium: dose-dependent inhibition by phytate. *J. Trace Elem. Med. Biol.,* **20**, 49-57.

Fridell F, Gadan K, Sundh H, Taranger GL, Glette J, Olsen RE, Sundell K, Evensen Ø (2007) Effect of hyperoxygenation and low water flow on the primary stress response and susceptibility of Atlantic salmon *Salmo salar* L. to experimental challenge with IPN virus. *Aquaculture,* **270**, 23-35.

Fritsch C, Vogel RF, Toelstede S (2015) Fermentation performance of lactic acid bacteria in different lupin substrates—influence and degradation ability of antinutritives and secondary plant metabolites. *J. Appl. Microbiol.,* **119**, 1075-1088.

Gallagher ML (1994) The use of soybean meal as a replacement for fish meal in diets for hybrid striped bass (*Morone saxatilis*×*M. chrysops*). *Aquaculture,* **126**, 119-127.

Gao Y-l, Wang C-s, Zhu Q-h, Qian G-y (2013) Optimization of solid-state fermentation with *Lactobacillus brevis* and *Aspergillus oryzae* for trypsin inhibitor degradation in soybean meal. *J. Integr. Agr.,* **12**, 869-876.

García-Mantrana I, Monedero V, Haros M (2015) Reduction of phytate in soy drink by fermentation with *Lactobacillus casei* expressing phytases from Bifidobacteria. *Plant Foods Hum. Nutr.*, **70**, 269-274.

Gatlin DM, Barrows FT, Brown P, Dabrowski K, Gaylord TG, Hardy RW, Herman E, Hu G, Krogdahl Å, Nelson R, Overturf K, Rust M, Sealey W, Skonberg D, J Souza E, Stone D, Wilson R, Wurtele E (2007) Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res.,* **38**, 551-579.

Gatlin DM, Poe WE, Wilson RP (1986) Effects of singular and combined dietary deficiencies of selenium and vitamin E on fingerling channel catfish (*Ictalurus punctatus*). *J. Nutr.,* **116**, 1061-1067.

Gatlin DM, Wilson RP (1984) Dietary selenium requirement of fingerling channel catfish. *J. Nutr.,* **114**, 627-633.

Glencross B (2009) The influence of soluble and insoluble lupin non-starch polysaccharides on the digestibility of diets fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **294**, 256-261.

Glencross B, Evans D, Dods K, McCafferty P, Hawkins W, Maas R, Sipsas S (2005) Evaluation of the digestible value of lupin and soybean protein concentrates and isolates when fed to rainbow trout, *Oncorhynchus mykiss*, using either stripping or settlement faecal collection methods. *Aquaculture,* **245**, 211-220.

Glencross B, Rutherford N (2010) Dietary strategies to improve the growth and feed utilization of barramundi, *Lates calcarifer* under high water temperature conditions. *Aquac. Nutr.,* **16**, 343-350.

Glencross BD (2006) The nutritional management of barramundi, *Lates calcarifer*– a review. *Aquac. Nutr.,* **12**, 291-309.

Glencross BD (2011) A comparison of the digestibility of diets and ingredients fed to rainbow trout (*Oncorhynchus mykiss*) or barramundi (*Lates calcarifer*) - the potential for inference of digestibility values among species. *Aquac. Nutr.,* **17**, e207-e215.

Glencross BD, Booth MA, Allan GL (2007a) A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. *Aquac. Nutr.,* **13**, 17-34.

Glencross BD, Boujard T, Kaushik SJ (2003a) Influence of oligosaccharides on the digestibility of lupin meals when fed to rainbow trout, *Oncorhynchus mykiss*. *Aquaculture,* **219**, 703-713.

Glencross BD, Carter CG, Duijster N, Evans DR, Dods K, McCafferty P, Hawkins WE, Maas R, Sipsas S (2004a) A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **237**, 333-346.

Glencross BD, Curnow J, Hawkins W, Kissil GWM, Peterson D (2003b) Evaluation of the feed value of a transgenic strain of the narrow-leaf lupin (*Lupinus angustifolius*) in the diet of the marine fish, *Pagrus auratus*. *Aquac. Nutr.,* **9**, 197-206.

Glencross BD, Evans DR, Hawkins W, Jones B (2004b) Evaluation of dietary inclusion of yellow lupin *(Lupinus luteus*) kernel meal on the growth, feed utilisation and tissue histology of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **235**, 411-422.

Glencross BD, Evans DR, Rutherford N, Hawkins W, McCafferty P, Dods K, Jones B, Harris D, Morton L, Sweetingham M, Sipsas S (2006a) The influence of the dietary inclusion of the alkaloid gramine, on rainbow trout (*Oncorhynchus mykiss*) growth, feed utilisation and gastrointestinal histology. *Aquaculture*, **253**, 512-522.

Glencross BD, Hawkins W (2004) A comparison of the digestibility of lupin *(Lupinus sp*.) kernel meals as dietary protein resources when fed to either, rainbow trout, *Oncorhynchus mykiss* or red seabream, *Pagrus auratus*. *Aquac. Nutr.*, **10**, 65-73.

Glencross BD, Hawkins W, Evans DR, Rutherford N, Dods K, McCafferty P, Sipsas S (2007b) Evaluation of the influence of drying process on the nutritional value of lupin protein concentrates when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **265**, 218-229.

Glencross BD, Hawkins WE, Evans D, Rutherford N, Dods K, Maas R, McCafferty P, Sipsas S (2006b) Evaluation of the nutritional value of prototype lupin protein concentrates when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **251**, 66-77.

Glencross BD, Hawkins WE, Evans DR, Rutherford N, McCafferty P, Dods K, Karopoulos M, Veitch C, Sipsas S, Buirchell B (2008) Variability in the composition of lupin (*Lupinus angustifolius*) meals influences their digestible nutrient and energy value when fed to rainbow trout *(Oncorhynchus mykiss*). *Aquaculture*, **277**, 220-230.

Glencross BD, Hawkins WE, Maas R, Karopoulos M, Hauler R (2010a) Evaluation of the influence of different species and cultivars of lupin kernel meal on the extrusion process, pellet properties and viscosity parameters of salmonid feeds. *Aquac. Nutr.*, **16**, 13-24.

Glencross BD, Hawkins WE, Veitch C, Dods K, McCafferty P, Hauler R (2007c) The influence of dehulling efficiency on the digestible value of lupin (*Lupinus angustifolius*) kernel meal when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquac. Nutr.*, **13**, 462-470.

Glencross BD, Rutherford N, Jones B (2011) Evaluating options for fishmeal replacement in diets for juvenile barramundi (*Lates calcarifer*). *Aquac. Nutr.*, **17**, e722-e732.

Glencross BD, Sweetingham M, Hawkins WE (2010b) A digestibility assessment of pearl lupin (*Lupinus mutabilis*) meals and protein concentrates when fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **303**, 59-64.

Globefish (2015) Fishmeal and Fish Oil - August 2015. In: *Market Reports: Fish Oil and Fishmeal*. FAO, Rome.

Goddard MR, Greig D (2015) *Saccharomyces cerevisiae*: a nomadic yeast with no niche? *FEMS Yeast Research,* **15**, fov009.

Gomes EF, Rema P, Gouveia A, Teles AO (1995) Replacement of fish meal by plant proteins in diets for rainbow trout (*Oncorhynchus mykiss*): Effect of the quality of the fishmeal based control diets on digestibility and nutrient balances. *Water Sci. Technol.,* **31**, 205-211.

Gouveia A, Davies SJ (2000) Inclusion of an extruded dehulled pea seed meal in diets for juvenile European sea bass (*Dicentrarchus labrax*). *Aquaculture,* **182**, 183-193.

Gratzfeld-Huesgen A (1998) Sensitive and reliable amino acid analysis in protein hydrolysates using the Agilent 1100 series HPLC. In: *Technical Note*. Agilent Technologies.

Grisdale-Helland B, Shearer KD, Gatlin Iii DM, Helland SJ (2008) Effects of dietary protein and lipid levels on growth, protein digestibility, feed utilization and body composition of Atlantic cod (*Gadus morhua*). *Aquaculture,* **283**, 156-162.

Gropper SAS, Smith JL, Groff JL (2009) *Advanced nutrition and human metabolism,* Wadsworth/Cengage Learning, Australia; United States.

Gui D, Liu W, Shao X, Xu W (2010) Effects of different dietary levels of cottonseed meal protein hydrolysate on growth, digestibility, body composition and serum biochemical indices in crucian carp (*Carassius auratus gibelio*). *Anim. Feed Sci. Tech.,* **156**, 112-120.

Güroy B, Şahin İ, Kayalı S, Mantoğlu S, Canan B, Merrifield DL, Davies SJ, Güroy D (2013) Evaluation of feed utilization and growth performance of juvenile striped catfish *Pangasianodon hypophthalmus* fed diets with varying inclusion levels of corn gluten meal. *Aquac. Nutr.,* **19**, 258-266.

Hamilton SJ (2004) Review of selenium toxicity in the aquatic food chain. *Sci. Total Environ.,* **326**, 1-31.

Hamilton SJ, Buhl KJ, Faerber NL, Bullard FA, Wiedmeyer RH (1990) Toxicity of organic selenium in the diet to chinook salmon. *Environ. Toxicol. Chem.,* **9**, 347-358.

Hamilton SJ, Holley KM, Buhl KJ, Bullard FA, Weston LK, McDonald SF (2002) Toxicity of selenium and other elements in food organisms to razorback sucker larvae. *Aquat. Toxicol.,* **59**, 253-281.

Han D, Xie S, Liu M, Xiao X, Liu H, Zhu X, Yang Y (2011) The effects of dietary selenium on growth performances, oxidative stress and tissue selenium concentration of gibel carp (*Carassius auratus gibelio*). *Aquac. Nutr.,* **17**, e741-e749.

Han YZ, Jiang ZQ, Ren TJ, Koshio S, Gao J, Komilus CF, Jiang BQ (2015) Effect of dietary fish oil replacement with palm oil on growth performance,

hematology and liver anti-oxidative enzymes of juvenile Japanese flounder *Paralichthys olivaceus* (Temminck & Schlegel, 1846). *J. Appl. Ichthyol.*, **31**, 518-524.

Handeland SO, Imsland AK, Stefansson SO (2008) The effect of temperature and fish size on growth, feed intake, food conversion efficiency and stomach evacuation rate of Atlantic salmon post-smolts. *Aquaculture,* **283**, 36-42.

Hao X, Ling Q, Hong F (2014) Effects of dietary selenium on the pathological changes and oxidative stress in loach (*Paramisgurnus dabryanus*). *Fish Physiol. Biochem.,* **40**, 1313-1323.

Hardy R, Oram L, Möller G (2010) Effects of dietary selenomethionine on cutthroat trout (*Oncorhynchus clarki bouvieri*) growth and reproductive performance over a life cycle. *Arch. Environ. Contam. Toxicol.,* **58**, 237-245.

Hardy RW (2010) Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. *Aquac. Res.,* **41**, 770-776.

Hardy RW, Barrows FT (2002) Diet formulation and manufacture In: *Fish Nutrition (3rd edition)* (ed. by Halver JE, Hardy RW). Academic Press Inc., San Diego, CA, USA, pp. 506-601.

Hargis Jr WJ (1991) Disorders of the eye in finfish. *Annu. Rev. Fish Dis.,* **1**, 95-117.

Harland BF, Morris ER (1995) Phytate: A good or a bad food component? *Nutr. Res.,* **15**, 733-754.

Hasan MR, Tacon AGJ, Metian M (2011) *Demand and supply of feed ingredients for farmed fish and crustaceans,* Food and Agriculture Organization of the United Nations, Rome.

Hassaan MS, Soltan MA, Abdel-Moez AM (2015) Nutritive value of soybean meal after solid state fermentation with *Saccharomyces cerevisiae* for Nile tilapia, *Oreochromis niloticus*. *Anim. Feed Sci. Tech.,* **201**, 89-98.

Hefnawy AEG, Tórtora-Pérez JL (2010) The importance of selenium and the effects of its deficiency in animal health. *Small Rumin. Res.,* **89**, 185-192.

Heikkinen J, Vielma J, Kemiläinen O, Tiirola M, Eskelinen P, Kiuru T, Navia-Paldanius D, von Wright A (2006) Effects of soybean meal based diet on growth performance, gut histopathology and intestinal microbiota of juvenile rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **261**, 259-268.

Hernandez C, Olvera-Novoa MA, Hardy RW, Hermosillo A, Reyes C, Gonzalez B (2010) Complete replacement of fish meal by porcine and poultry by-product meals in practical diets for fingerling Nile tilapia *Oreochromis niloticus*: digestibility and growth performance. *Aquac. Nutr.,* **16**, 44-53.

Hernández MD, Martínez FJ, Jover M, García García B (2007) Effects of partial replacement of fish meal by soybean meal in sharpsnout seabream (*Diplodus puntazzo*) diet. *Aquaculture*, **263**, 159-167.

Hertrampf JW, Piedad-Pascual F (2000) *Handbook on Ingredients for Aquaculture Feeds*, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hilton JW, Hodson PV, Slinger SJ (1980) The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *J. Nutr.*, **110**, 2527-2535.

Hixson SM, Parrish CC, Wells JS, Winkowski EM, Anderson DM, Bullerwell CN (2016) Inclusion of camelina meal as a protein source in diets for farmed salmonids. *Aquac. Nutr.*, **22**, 615-630.

Hoffmann PR, Berry MJ (2008) The influence of selenium on immune responses. *Mol. Nutr. Food Res.*, **52**, 1273-1280.

Hossain MA, Jauncey K (1993) The effects of varying dietary phytic acid, calcium and magnesium levels on the nutrition of common carp, *Cyprinus carpio*. In: *The 4th International Symposium on Fish Nutrition and Feeding* (ed INRA), Biarritz, France, pp. 705-715.

Hotz C, Gibson RS (2007) Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *J. Nutr.*, **137**, 1097-1100.

Hua K, Bureau DP (2012) Exploring the possibility of quantifying the effects of plant protein ingredients in fish feeds using meta-analysis and nutritional model simulation-based approaches. *Aquaculture*, **356–357**, 284-301.

Huntington TC, Hasan MR (2009) Fish as feed inputs for aquaculture - practices, sustainability and implications: a global synthesis. In: *Fish as feed inputs for aquaculture: practices, sustainability and implications* (ed. by Hasan MR, Halwart M). FAO, Rome, pp. 1-61.

Hussain SM, Afzal M, Nasir S, Javid A, Azmat H, Mamoona Makhdoom S, Shah SZH, Hussain M, Mustafa I, Iqbal M (2015) Role of phytase supplementation in improving nutrient digestibility and growth performance for *Labeo rohita* fingerlings fed on canola meal-based diet. *J. Appl. Anim. Res.*, 1-7.

Iguchi Ki, Ogawa K, Nagae M, Ito F (2003) The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). *Aquaculture*, **220**, 515-523.

Ilham I, Fotedar R (2016) Growth, antioxidant capacity and muscle histochemistry of yellowtail kingfish (*Seriola lalandi* Valenciennes 1883): Selenium and temperature interaction. *Anim. Feed Sci. Tech.*, **217**, 76-86.

Ilham I, Fotedar R, Munilkumar S (2016a) Effects of organic selenium supplementation on growth, glutathione peroxidase activity and histopathology in juvenile barramundi (*Lates calcarifer* Bloch 1970) fed high lupin meal-based diets. *Aquaculture*, **457**, 15-23.

Ilham I, Fotedar R, Suyasa IN (2016b) Use of organic selenium supplements in soybean meal-based diets for juvenile yellowtail kingfish (*Seriola lalandi*). *Int. J Food Nutr. Sci.*, **3**, 1-11.

Ilham I, Siddik MAB, Fotedar R (2016c) Effects of organic selenium supplementation on growth, accumulation, haematology and histopathology of juvenile barramundi (*Lates calcarifer*) fed high soybean meal diets. *Biol. Trace Elem. Res.,* **174**, 436-447.

Imsland AK, Sunde LM, Folkvord A, Stefansson SO (1996) The interaction of temperature and fish size on growth of juvenile turbot. *J. Fish Biol.,* **49**, 926-940.

Iwata M, Komatsu S, Collie NL, Nishioka RS, Bern HA (1987) Ocular cataract and seawater adaptation in salmonids. *Aquaculture,* **66**, 315-327.

Jahanbakhshi A, Imanpoor MR, Taghizadeh V, Shabani A (2013) Hematological and serum biochemical indices changes induced by replacing fish meal with plant protein (sesame oil cake and corn gluten) in the great sturgeon (*Huso huso*). *Comp. Clin. Path.,* **22**, 1087-1092.

Janz DM (2011) 7 - Selenium. In: *Fish Physiology* (ed. by Chris M. Wood APF, Colin JB). Academic Press, London, pp. 327-374.

Jaramillo Jr F, Peng LI, Gatlin Iii DM (2009) Selenium nutrition of hybrid striped bass (*Morone chrysops* × *M. saxatilis*) bioavailability, toxicity and interaction with vitamin E. *Aquac. Nutr.,* **15**, 160-165.

Jeong JS, Park JW, Lee SI, Kim IH (2015) Apparent ileal digestibility of nutrients and amino acids in soybean meal, fish meal, spray-dried plasma protein and fermented soybean meal to weaned pigs. *Anim. Sci. J.*, n/a-n/a.

Jirsa D, Barrows FT, Hardy RW, Drawbridge M (2015) Alternative protein blends as a replacement for fish meal in diets for white seabass, *Atractoscion nobilis*. *Aquac. Nutr.,* **21**, 861-867.

Job S (2011) Barramundi Aquaculture. In: *Recent Advances and New Species in Aquaculture*. Wiley-Blackwell, pp. 199-230.

Jobling M, Gomes E, Dias J (2007) Feed types, manufacture and ingredients. In: *Food Intake in Fish*. Blackwell Science Ltd, pp. 25-48.

Kader MA, Bulbul M, Koshio S, Ishikawa M, Yokoyama S, Nguyen BT, Komilus CF (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**, 109-116.

Kate SH (2013) Infectious Diseases of Asian Seabass and Health Management. In: *Biology and Culture of Asian Seabass Lates Calcarifer*. CRC Press, pp. 103-136.

Katersky RS, Carter CG (2005) Growth efficiency of juvenile barramundi, *Lates calcarifer*, at high temperatures. *Aquaculture,* **250**, 775-780.

Katersky RS, Carter CG (2007) A preliminary study on growth and protein synthesis of juvenile barramundi, *Lates calcarifer* at different temperatures. *Aquaculture*, **267**, 157-164.

Katersky RS, Carter CG (2009) Growth and protein synthesis of barramundi, *Lates calcarifer*, fed lupin as a partial protein replacement. *Comparative Biochemistry and Physiology. Part A: Molecular and Integrative Physiology*, **152**, 513-517.

Katya K, Yun Y-h, Yun H, Lee J-Y, Bai SC (2014) Effects of dietary fermented by-product of mushroom, *Pleurotus ostreatus*, as an additive on growth, serological characteristics and nonspecific immune responses in juvenile Amur catfish, *Silurus asotus*. *Aquac. Res.*, n/a-n/a.

Kaushik SJ, Cravedi JP, Lalles JP, Sumpter J, Fauconneau B, Laroche M (1995) Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, **133**, 257-274.

Kenari AA, Mahmoudi N, Soltani M, Abediankenari S (2013) Dietary nucleotide supplements influence the growth, haemato-immunological parameters and stress responses in endangered Caspian brown trout (*Salmo trutta caspius* Kessler, 1877). *Aquac. Nutr.*, **19**, 54-63.

Khattab RY, Arntfield SD (2009) Nutritional quality of legume seeds as affected by some physical treatments 2. Antinutritional factors. *LWT - Food Sci. Tech.*, **42**, 1113-1118.

Kikuchi K (1999) Use of defatted soybean meal as a substitute for fish meal in diets of Japanese flounder (*Paralichthys olivaceus*). *Aquaculture*, **179**, 3-11.

Kim J-D, Tibbetts SM, Milley JE, Lall SP (2007) Effect of the incorporation level of dehulled soybean meal into test diet on apparent digestibility coefficients for protein and energy by juvenile haddock, *Melanogrammus aeglefinus* L. *Aquaculture*, **267**, 308-314.

Kim J-H, Kang J-C (2014) The selenium accumulation and its effect on growth, and haematological parameters in red sea bream, *Pagrus major*, exposed to waterborne selenium. *Ecotoxicol. Environ. Saf.*, **104**, 96-102.

Kim K-D, Kim K-M, Kim K-W, Jin Kang Y, Lee S-M (2006) Influence of lipid level and supplemental lecithin in diet on growth, feed utilization and body composition of juvenile flounder (*Paralichthys olivaceus*) in suboptimal water temperatures. *Aquaculture*, **251**, 484-490.

Kim S-S, Pham M, Kim K-W, Son M-H, Lee K-J (2010) Effects of microbial fermentation of soybean on growth performances, phosphorus availability, and antioxidant activity in diets for juvenile olive flounder (Paralichthys olivaceus). *Food Sci. Biotechnol.*, **19**, 1605-1610.

Kiremidjian-Schumacher L, Stotzky G (1987) Selenium and immune responses. *Environmental Research Series,* **42**, 277-303.

Kissil GW, Lupatsch I, Higgs DA, Hardy RW (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. *Aquac. Res.*, **31**, 595-601.

Kohen R, Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.*, **30**, 620-650.

Köhrle J, Brigelius-Flohé R, Böck A, Gärtner R, Meyer O, Flohé L (2000) Selenium in biology: facts and medical perspectives. *Biol. Chem.*, **381**, 849-864.

Kokou F, Rigos G, Henry M, Kentouri M, Alexis M (2012) Growth performance, feed utilization and non-specific immune response of gilthead sea bream (*Sparus aurata* L.) fed graded levels of a bioprocessed soybean meal. *Aquaculture*, **364–365**, 74-81.

Köprücü K (2012) Variability in the chemical composition of different cottonseed and sunflower meals influences their digestibility when fed to grass carp (*Ctenopharyngodon idella*). *Aquac. Nutr.*, **18**, 662-672.

Krogdahl Å, Bakke-McKellep AM, 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.). *Aquac. Nutr.*, **9**, 361-371.

Krogdahl Å, Penn M, Thorsen J, Refstie S, Bakke AM (2010) Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquac. Res.*, **41**, 333-344.

Król E, Douglas A, Tocher DR, Crampton VO, Speakman JR, Secombes CJ, Martin SAM (2016) Differential responses of the gut transcriptome to plant protein diets in farmed Atlantic salmon. *BMC Genomics*, **17**, 156.

Kucukbay FZ, Yazlak H, Karaca I, Sahin N, Tuzcu M, Cakmak MN, Sahin K (2009) The effects of dietary organic or inorganic selenium in rainbow trout (*Oncorhynchus mykiss*) under crowding conditions. *Aquac. Nutr.*, **15**, 569-576.

Kumar V, Sinha AK, Makkar HPS, De Boeck G, Becker K (2012) Phytate and phytase in fish nutrition. *J. Anim. Physiol. Anim. Nutr. (Berl.)*, **96**, 335-364.

Kwanyuen P, Burton JW (2005) A simple and rapid procedure for phytate determination in soybeans and soy products. *J. Am. Oil Chem. Soc.*, **82**, 81-85.

Laining A, Traifalgar RF, Thu M, Komilus CF, Kader MA, Koshio S, Ishikawa M, Yokoyama S (2010) Influence of dietary phytic acid on growth, feed intake,

and nutrient utilization in juvenile Japanese flounder, *Paralichthys olivaceus*. *J. World Aquacult. Soc.,* **41**, 746-755.

Lall SP (2003) 5 - The Minerals. In: *Fish Nutrition (Third Edition)* (ed. by John EH, Ronald WH). Academic Press, San Diego, pp. 259-308.

Lanari D, D'Agaro E (2005) Alternative plant protein sources in sea bass diets. *Italian Journal of Animal Science,* **4**, 365-374.

Laporte J, Trushenski J (2012) Production performance, stress tolerance and intestinal integrity of sunshine bass fed increasing levels of soybean meal. *J. Anim. Physiol. Anim. Nutr. (Berl.),* **96**, 513-526.

Le K, Fotedar R (2013a) Dietary selenium requirement of yellowtail kingfish (*Seriola lalandi*). *Agricult. Sci.,* **4**, 68-75.

Le KT, Dao TTT, Fotedar R, Partrigde GJ (2014a) Effects of variation in dietary contents of selenium and vitamin E on growth and physiological and haematological responses of yellowtail kingfish, *Seriola lalandi*. *Aquac. Int.,* **22**, 435-446.

Le KT, Fotedar R (2013b) Dietary selenium requirement of yellowtail kingfish (*Seriola lalandi*). *Agricult. Sci.,* **4**, 68-75.

Le KT, Fotedar R (2014a) Bioavailability of selenium from different dietary sources in yellowtail kingfish (*Seriola lalandi*). *Aquaculture,* **420–421**, 57-62.

Le KT, Fotedar R (2014b) Immune responses to *Vibrio anguillarum* in yellowtail kingfish, *Seriola lalandi*, fed selenium supplementation. *J. World Aquacult. Soc.,* **45**, 138-148.

Le KT, Fotedar R (2014c) Toxic effects of excessive levels of dietary selenium in juvenile yellowtail kingfish (*Seriola lalandi*). *Aquaculture,* **433**, 229-234.

Le KT, Fotedar R, Partridge GJ (2014b) Selenium and vitamin E interaction in the nutrition of yellowtail kingfish (*Seriola lalandi*): physiological and immune responses. *Aquac. Nutr.,* **20**, 303-313.

Lee S, Nambi RW, Won S, Katya K, Bai SC (2016) Dietary selenium requirement and toxicity levels in juvenile Nile tilapia, *Oreochromis niloticus*. *Aquaculture,* **464**, 153-158.

Lemly AD (1993) Metabolic stress during winter increases the toxicity of selenium to fish. *Aquat. Toxicol.,* **27**, 133-158.

Lemly AD (1996) Winter stress syndrome: an important consideration for hazard assessment of aquatic pollutants. *Ecotoxicol. Environ. Saf.,* **34**, 223-227.

Lemly AD (1997) A teratogenic deformity index for evaluating impacts of selenium on fish populations. *Ecotoxicol. Environ. Saf.,* **37**, 259-266.

Lemly AD (2002) Symptoms and implications of selenium toxicity in fish: the Belews Lake case example. *Aquat. Toxicol.,* **57**, 39-49.

Lescure A, Rederstorff M, Krol A, Guicheney P, Allamand V (2009) Selenoprotein function and muscle disease. *BBA-Gen. Subjects*, **1790**, 1569-1574.

Li X-F, Tian H-Y, Zhang D-D, Jiang G-Z, Liu W-B (2014) Feeding frequency affects stress, innate immunity and disease resistance of juvenile blunt snout bream *Megalobrama amblycephala*. *Fish Shellfish Immunol.,* **38**, 80-87.

Lim S-J, Lee K-J (2009) Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. *Aquaculture,* **290**, 283-289.

Lim S-J, Lee K-J (2011) A microbial fermentation of soybean and cottonseed meal increases antioxidant activity and gossypol detoxification in diets for Nile tilapia, *Oreochromis niloticus*. *J. World Aquacult. Soc.,* **42**, 494-503.

Lim SR, Choi SM, Wang XJ, Kim KW, Shin IS, Min TS, Bai SC (2004) Effects of dehulled soybean meal as a fish meal replacer in diets for fingerling and growing Korean rockfish *Sebastes schlegeli*. *Aquaculture,* **231**, 457-468.

Lin H, Chen X, Chen S, Zhuojia L, Huang Z, Niu J, Wu K, Lu X (2012) Replacement of fish meal with fermented soybean meal in practical diets for pompano *Trachinotus ovatus*. *Aquac. Res.,* **44**, 151-156.

Lin S, Luo L (2011) Effects of different levels of soybean meal inclusion in replacement for fish meal on growth, digestive enzymes and transaminase activities in practical diets for juvenile tilapia, *Oreochromis niloticus* × *O. aureus*. *Anim. Feed Sci. Tech.,* **168**, 80-87.

Lin Y-H, Shiau S-Y (2005) Dietary selenium requirements of juvenile grouper, *Epinephelus malabaricus*. *Aquaculture,* **250**, 356-363.

Lin Y-H, Shiau S-Y (2009) Mutual sparing of dietary requirements for alpha-tocopherol and selenium in grouper, *Epinephelus malabaricus*. *Aquaculture,* **294**, 242-245.

Lindh U (2013) Biological functions of the elements. In: *Essentials of Medical Geology* (ed by Selinus O). Springer Netherlands, pp. 129-177.

Liu K, Wang XJ, Ai Q, Mai K, Zhang W (2010) Dietary selenium requirement for juvenile cobia, *Rachycentron canadum* L. *Aquac. Res.,* **41**, e594-e601.

López-Albors O, Gil F, Ramírez-Zarzosa G, Latorre R, García-Alcázar A, Abellán E, Blanco A, Vázquez JM, Moreno F (1995) Early muscle injuries in a standard reared stock of sea bass *Dicentrarchus labrax*, (L.). *Aquaculture,* **138**, 69-76.

López-Alonso M (2012) Trace Minerals and Livestock: Not Too Much Not Too Little. *ISRN Vet. Sci.,* **2012**, 18.

López LM, Flores-Ibarra M, Bañuelos-Vargas I, Galaviz MA, True CD (2015) Effect of fishmeal replacement by soy protein concentrate with taurine supplementation on growth performance, hematological and biochemical status, and liver histology of totoaba juveniles (*Totoaba macdonaldi*). *Fish Physiol. Biochem.,* **41**, 921-936.

Lorentzen M, Maage A, Julshamn K (1994) Effects of dietary selenite or selenomethionine on tissue selenium levels of Atlantic salmon (*Salmo salar*). *Aquaculture*, **121**, 359-367.

Lucas MM, Stoddard FL, Annicchiarico P, Frías J, Martínez-Villaluenga C, Sussmann D, Duranti M, Seger A, Zander PM, Pueyo JJ (2015) The future of lupin as a protein crop in Europe. *Front. Plant Sci.*, **6**, 705.

Luna LG (1968) Manual of histological staining methods of the Armed Forces Institute of Pathology, 3rd Edition. McGraw-Hill Book Company, New York.

Lund B, Baird-Parker TC (2000) *Microbiological Safety and Quality of Food,* Aspen Publisher, Gaithersburg, MD.

Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. *Aquat. Toxicol.,* **101**, 13-30.

Lushchak VI, Bagnyukova TV (2006) Temperature increase results in oxidative stress in goldfish tissues. 2. Antioxidant and associated enzymes. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.,* **143**, 36-41.

Lyons MP, Papazyan TT, Surai PF (2007) Selenium in food chain and animal nutrition: lessons from nature - Review. *Asian-Australas. J. Anim. Sci.,* **20**, 1135-1155.

Maher WA (1985) Characteristics of selenium in marine animals. *Mar. Pollut. Bull.,* **16**, 33-34.

Martin TL, Huey RB (2008) Why "suboptimal" is optimal: Jensen's inequality and ectotherm thermal preferences. *Am. Nat.,* **171**, E102-118.

Maynard LA, Loosli JK (1969) *Animal Nutrition,* McGraw Hill Book Company, New York.

McCord JM (2000) The evolution of free radicals and oxidative stress. *Am. J. Med.,* **108**, 652-659.

McKenzie RC, Arthur JR, Beckett GJ (2002) Selenium and the regulation of cell signaling, growth, and survival: Molecular and mechanistic mspects. *Antioxid. Redox Signal.,* **4**, 339–351.

McLeay DJ, Gordon MR (1977) Leucocrit: a simple hematological technique for measuring acute stress in salmonid fish, including stressful concentrations of pulpmill effluent. *J. Fish Res. Board Can.,* **34**, 2156-2163.

Médale F, Boujard T, Vallée F, Blanc D, Mambrini M, Roem A, Kaushik SJ (1998) Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout (*Oncorhynchus mykiss*) fed increasing dietary levels of soy protein concentrate. *Aquat. Living Resour.,* **11**, 239-246.

Meyer J, Moulis J-M, Gaillard J, Lutz M (1992) Replacement of sulfur by selenium in iron-sulfur proteins. In: *Adv. Inorg. Chem.* (ed. by Cammack R, Skyes AG). Academic Press, San Diego, pp. 73-116.

Misra S, Peak D, Chen N, Hamilton C, Niyogi S (2012) Tissue-specific accumulation and speciation of selenium in rainbow trout (*Oncorhynchus mykiss*) exposed to elevated dietary selenomethionine. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.,* **155**, 560-565.

Moghadaszadeh B, Beggs AH (2006) Selenoproteins and their impact on human health through diverse physiological pathways. *Physiology,* **21**, 307-315.

Molina-Poveda C, Lucas M, Jover M (2013) Evaluation of the potential of Andean lupin meal (*Lupinus mutabilis* Sweet) as an alternative to fish meal in juvenile *Litopenaeus vannamei* diets. *Aquaculture,* **410–411**, 148-156.

Moran D, Pether SJ, Lee PS (2009) Growth, feed conversion and faecal discharge of yellowtail kingfish (*Seriola lalandi*) fed three commercial diets. *N. Z. J. Mar. Freshwater Res.,* **43**, 917-927.

Moren M, Waagbø R, Hamre K (2011) Micronutrients. In: *Larval Fish Nutrition* (ed by Holt GJ). Wiley-Blackwell, Chicester, West Sussex, UK, pp. 117-149.

Mukhopadhyay BN, Ray AK (1999) Utilization of copra meal in the formulation of compound diets for rohu, *Labeo rohita*, fingerlings. *J. Appl. Ichthyol.,* **15**, 127-131.

Murashita K, Akimoto A, Iwashita Y, Amano S, Suzuki N, Matsunari H, Furuita H, Sugita T, Yamamoto T (2013) Effects of biotechnologically processed soybean meals in a nonfishmeal diet on growth performance, bile acid status, and morphological condition of the distal intestine and liver of rainbow trout *Oncorhynchus mykiss*. *Fish. Sci.,* **79**, 447-457.

Muzinic LA, Thompson KR, Morris A, Webster CD, Rouse DB, Manomaitis L (2004) Partial and total replacement of fish meal with soybean meal and brewer's grains with yeast in practical diets for Australian red claw crayfish *Cherax quadricarinatus*. *Aquaculture,* **230**, 359-376.

Mwachireya, Beames, Higgs, Dosanjh (1999) Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout *Oncorhynchus mykiss* (Walbaum) held in fresh water. *Aquac. Nutr.,* **5**, 73-82.

Nakano T, Kameda M, Shoji Y, Hayashi S, Yamaguchi T, Sato M (2014) Effect of severe environmental thermal stress on redox state in salmon. *Redox Biology,* **2**, 772-776.

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. *Nature,* **405**, 1017-1024.

Nelson JS (1994) *Fishes of the World,* John Wiley & Sons New York.

Nengas I, Alexis MN, Davies SJ (1996) Partial substitution of fishmeal with soybean meal products and derivatives in diets for the gilthead sea bream *Sparus aurata* (L.). *Aquac. Res.,* **27**, 147-156.

Neve J, Richard M-J (1994) Concenpts and Effects of Intervention Studies with Antioxidative Trace elements in Humans. In: *Trace Elements and Free Radicals in Oxidative Diseases* (ed. by Favier AE, Neve J, Faure P). The American Oil Chemists Society Press, Champaign, Illinois, pp. 310.

Ng W-K, Lim H-A, Lim S-L, Ibrahim C-O (2002) Nutritive value of palm kernel meal pretreated with enzyme or fermented with *Trichoderma koningii* (Oudemans) as a dietary ingredient for red hybrid tilapia (*Oreochromis sp*.). *Aquac. Res.*, **33**, 1199-1207.

Ngo DT, Pirozzi I, Glencross B (2015) Digestibility of canola meals in barramundi (Asian seabass; *Lates calcarifer*). *Aquaculture,* **435**, 442-449.

Ngo DT, Wade NM, Pirozzi I, Glencross BD (2016) Effects of canola meal on growth, feed utilisation, plasma biochemistry, histology of digestive organs and hepatic gene expression of barramundi (Asian seabass; *Lates calcarifer*). *Aquaculture*, **464**, 95-105.

Nguyen HP, Khaoian P, Fukada H, Nakamori T, Furuta H, Masumoto T (2011) Effects of different soybean proteins on lipid digestion and growth of yellowtail Seriola quinqueradiata. *Fish. Sci.,* **77**, 357-365.

Nguyen HP, Khaoian P, Fukada H, Suzuki N, Masumoto T (2015) Feeding fermented soybean meal diet supplemented with taurine to yellowtail *Seriola quinqueradiata* affects growth performance and lipid digestion. *Aquac. Res.,* **46**, 1101-1110.

NRC (1993) *Nutrient Requirements of Fish,* The National Academies Press, Washington DC.

NRC (2011) *Nutrient requirements of fish and shrimp,* National Academies Press, Washington, D.C.

Nugroho RA, Fotedar R (2013) Dietary organic selenium improves growth, survival and resistance to *Vibrio mimicus* in cultured marron, *Cherax cainii* (Austin, 2002). *Fish Shellfish Immunol.*, **35**, 79-85.

OECD/FAO (2014) *OECD-FAO Agricultural Outlook 2014-2023,* OECD Publishing, Paris.

Ogle R, Knight A (1989) Effects of elevated foodborne selenium on growth and reproduction of the fathead minnow (*Pimephales promelas*). *Arch. Environ. Contam. Toxicol.,* **18**, 795-803.

Ogle RS, Maier KJ, Kiffney P, Williams MJ, Brasher A, Melton LA, Knight AW (1988) Bioaccumulation of selenium in aquatic ecosystems. *Lake Reserv. Manag.,* **4**, 165-173.

Olsen RE, Hansen A-C, Rosenlund G, Hemre G-I, Mayhew TM, Knudsen DL, Tufan Eroldoğan O, Myklebust R, Karlsen Ø (2007) Total replacement of fish meal with plant proteins in diets for Atlantic cod (*Gadus morhua* L.) II — Health aspects. *Aquaculture,* **272**, 612-624.

Olsen RL, Hasan MR (2012) A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends Food Sci. Tech.,* **27**, 120-128.

Omnes MH, Silva FCP, Moriceau J, Aguirre P, Kaushik S, Gatesoupe FJ (2015) Influence of lupin and rapeseed meals on the integrity of digestive tract and organs in gilthead seabream (*Sparus aurata* L.) and goldfish (*Carassius auratus* L.) juveniles. *Aquac. Nutr.,* **21**, 223-233.

Ornsrud R, Lorentzen M (2002) Bioavailability of selenium from raw or cured selenomethionine-enriched fillets of Atlantic salmon (*Salmo salar*) assessed in selenium-deficient rats. *Br. J. Nutr.,* **87**, 13-20.

Ostaszewska T, Dabrowski K, Palacios ME, Olejniczak M, Wieczorek M (2005) Growth and morphological changes in the digestive tract of rainbow trout (*Oncorhynchus mykiss*) and pacu (*Piaractus mesopotamicus*) due to casein replacement with soybean proteins. *Aquaculture,* **245**, 273-286.

Ouraji H, Zaretabar A, Rahmani H (2013) Performance of rainbow trout (*Oncorhynchus mykiss*) fingerlings fed diets containing different levels of faba bean (*Vicia faba*) meal. *Aquaculture,* **416–417**, 161-165.

Pacini N, Abete MC, Dörr AJM, Prearo M, Natali M, Elia AC (2012) Detoxifying response in juvenile tench fed by selenium diet. *Environ. Toxicol. Pharmacol.,* **33**, 46-52.

Pacini N, Elia AC, Abete MC, Dorr AJ, Brizio P, Gasco L, Righetti M, Prearo M (2013) Antioxidant response versus selenium accumulation in the liver and kidney of the Siberian sturgeon (*Acipenser baeri*). *Chemosphere,* **93**, 2405-2412.

Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.,* **70**, 158-169.

Pamplona R, Costantini D (2011) Molecular and structural antioxidant defenses against oxidative stress in animals. *Am. J. Physiol. - Reg. Integr. Comp. Physiol.,* **301**, R843-R863.

Papatryphon E, Howell RA, Soares JHJ (1999) Growth and mineral absorption by striped bass *Morone saxatilis* fed a plant feedstuff based diet supplemented with phytase. *J. World Aquacult. Soc.,* **30**, 161-173.

Paripatananont T, Lovell RT (1998) Minerals: Comparative digestibilities of chelated and inorganic trace minerals in channel catfish (*Ictalurus punctatus*) diets. *Aquaculture,* **161**, 381.

Parrilla-Taylor DP, Zenteno-Savín T (2011) Antioxidant enzyme activities in Pacific white shrimp (*Litopenaeus vannamei*) in response to environmental hypoxia and reoxygenation. *Aquaculture,* **318**, 379-383.

Parrilla-Taylor DP, Zenteno-Savín T, Magallón-Barajas FJ (2013) Antioxidant enzyme activity in pacific whiteleg shrimp (*Litopenaeus vannamei*) in response to infection with white spot syndrome virus. *Aquaculture,* **380–383**, 41-46.

Paul H, Chris C, Marty P (2013) Farming of Barramundi/Asian Seabass. In: *Biology and Culture of Asian Seabass Lates calcarifer*. CRC Press, pp. 259-272.

Pedrero Z, Madrid Y (2009) Novel approaches for selenium speciation in foodstuffs and biological specimens: A review. *Anal. Chim. Acta,* **634**, 135-152.

Peng M, Xu W, Ai Q, Mai K, Liufu Z, Zhang K (2013) Effects of nucleotide supplementation on growth, immune responses and intestinal morphology in juvenile turbot fed diets with graded levels of soybean meal (*Scophthalmus maximus* L.). *Aquaculture,* **392-395**, 51-58.

Penglase S, Hamre K, Rasinger JD, Ellingsen S (2014) Selenium status affects selenoprotein expression, reproduction, and F1 generation locomotor activity in zebrafish (*Danio rerio*). *Br. J. Nutr.,* **FirstView**, 1-14.

Penglase S, Nordgreen A, van der Meeren T, Olsvik PA, Sæle Ø, Sweetman JW, Baeverfjord G, Helland S, Hamre K (2010) Increasing the level of selenium in rotifers (*Brachionus plicatilis* 'Cayman') enhances the mRNA expression and activity of glutathione peroxidase in cod (*Gadus morhua* L.) larvae. *Aquaculture,* **306**, 259-269.

Penn MH, Bendiksen EÅ, Campbell P, Krogdahl Å (2011) High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (*Salmo salar* L.). *Aquaculture,* **310**, 267-273.

Pereira TG, Oliva-Teles A (2004) Evaluation of micronized lupin seed meal as an alternative protein source in diets for gilthead sea bream *Sparus aurata* L. juveniles. *Aquac. Res.,* **35**, 828-835.

Petterson DS (2004) Lupin In: *Encyclopedia of Grain Science*. Elsevier, Oxford, pp. 166-174.

Pirozzi I, Booth MA (2009) The routine metabolic rate of mulloway (*Argyrosomus japonicus*: Sciaenidae) and yellowtail kingfish (*Seriola lalandi*: Carangidae)

acclimated to six different temperatures. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **152**, 586-592.

Plaipetch P, Yakupitiyage A (2014) Effect of replacing soybean meal with yeast-fermented canola meal on growth and nutrient retention of Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). *Aquac. Res.*, **45**, 1744-1753.

Poortenaar CW, Hooker SH, Sharp N (2001) Assessment of yellowtail kingfish (*Seriola lalandi lalandi*) reproductive physiology, as a basis for aquaculture development. *Aquaculture,* **201**, 271-286.

Poston HA, Combs GF, Leibovitz L (1976a) Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): gross, histological and biochemical signs. *J. Nutr.*, **106**, 892-904.

Poston HA, Combs GF, Leibovitz L (1976b) Vitamin E and selenium interrelations in the diet of Atlantic salmon (*Salmo salar*): gross, histological and biochemical signs. *J. Nutr.*, **106**, 892-904.

Prabhu PAJ, Schrama JW, Kaushik SJ (2014) Mineral requirements of fish: a systematic review. *Rev. Aquac.*, **6**, 1-48.

Pratoomyot J, Bendiksen EÅ, Bell JG, Tocher DR (2010) Effects of increasing replacement of dietary fishmeal with plant protein sources on growth performance and body lipid composition of Atlantic salmon (*Salmo salar* L.). *Aquaculture*, **305**, 124-132.

Raes K, Knockaert D, Struijs K, Van Camp J (2014) Role of processing on bioaccessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. *Trends Food Sci. Tech.*, **37**, 32-41.

Rayner CJ (1985) Protein hydrolysis of animal feeds for amino acid content. *J. Agric. Food Chem.*, **33**, 722-725.

Reddy NR, Pierson MD (1994) Reduction in antinutritional and toxic components in plant foods by fermentation. *Food Res. Int.*, **27**, 281-290.

Reddy NR, Pierson MD, Sathe SK, Salunkhe DK (1989) *Phytates in Cereals and Legumes*, Taylor & Francis.

Refstie S, Førde-Skjærvik O, Rosenlund G, Rørvik K-A (2006a) Feed intake, growth, and utilisation of macronutrients and amino acids by 1- and 2-year old Atlantic cod (*Gadus morhua*) fed standard or bioprocessed soybean meal. *Aquaculture*, **255**, 279-291.

Refstie S, Glencross BD, Landsverk T, Sørensen M, Lilleeng E, Hawkins W, Krogdahl Å (2006b) Digestive function and intestinal integrity in Atlantic salmon (*Salmo salar*) fed kernel meals and protein concentrates made from yellow or narrow-leafed lupins. *Aquaculture*, **261**, 1382-1395.

Refstie S, Sahlström S, Bråthen E, Baeverfjord G, Krogedal P (2005) Lactic acid fermentation eliminates indigestible carbohydrates and antinutritional factors

in soybean meal for Atlantic salmon (*Salmo salar*). *Aquaculture,* **246**, 331-345.

Refstie S, Storebakken T (2001) Vegetable protein sources for carnivorous fish: potential and challenges. *Recent Adv. Anim. Nutr. Aust.,* **13**, 195-203.

Refstie S, Storebakken T, Roem AJ (1998) Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. *Aquaculture,* **162**, 301-312.

Regost C, Arzel J, Kaushik SJ (1999) Partial or total replacement of fish meal by corn gluten meal in diet for turbot (*Psetta maxima*). *Aquaculture,* **180**, 99-117.

Ribeiro ARA, Ribeiro L, SÆLe Ø, Hamre K, Dinis MT, Moren M (2012) Selenium supplementation changes glutathione peroxidase activity and thyroid hormone production in Senegalese sole (*Solea senegalensis*) larvae. *Aquac. Nutr.*, **18**, 559-567.

Richardson NL, Higgs DA, Beames RM, McBride JR (1985) Influence of dietary calcium, phosphorus, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *J. Nutr.*, **115**, 553-567.

Rider SA, Davies SJ, Jha AN, Fisher AA, Knight J, Sweetman JW (2009) Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (*Oncorhynchus mykiss*): Implications on selenium status and health responses. *Aquaculture,* **295**, 282-291.

Rimmer MA, John Russell D (1998) 14 - Aspects of the biology and culture of *Lates calcarifer*. In: *Tropical Mariculture* (ed by Silva SSD). Academic Press, London, pp. 449-476.

Rinchard J, Lee KJ, Czesny S, Ciereszko A, Dabrowski K (2003) Effect of feeding cottonseed meal-containing diets to broodstock rainbow trout and their impact on the growth of their progenies. *Aquaculture,* **227**, 77-87.

Robaina L, Izquierdo MS, Moyano FJ, Socorro J, Vergara JM, Montero D, Fernández-Palacios H (1995) Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutritional and histological implications. *Aquaculture,* **130**, 219-233.

Rodger HD, Murphy TM, Drinan EM, Rice DA (1991) Acute skeletal myopathy in farmed Atlantic salmon *Salmo salar*. *Dis. Aquat. Organ.,* **12**, 17-23.

Root RW (1931) The respiratory function of the blood of marine fishes. *Biol. Bull.,* **61**, 427-456.

Rossi Jr W, Tomasso JR, Gatlin III DM (2015) Production performance and non-specific immunity of cage-raised red drum, *Sciaenops ocellatus*, fed soybean-based diets. *Aquaculture,* **443**, 84-89.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science,* **179**, 588-590.

Sajjadi M, Carter CG (2004) Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (*Salmo salar* L.) fed a canola-meal-based diet. *Aquaculture,* **240**, 417-431.

Salim ur R, Awan JA, Anjum FM, Randhawa M (2014) Antinutrients and Toxicity in Plant-based Foods. In: *Practical Food Safety* (ed. by Bhat R, Gómez-López VM). John Wiley & Sons, Ltd, pp. 311-339.

Salini MJ, Adams LR (2014) Growth performance, nutrient utilisation and digestibility by Atlantic salmon (*Salmo salar* L.) fed Tasmanian grown white *(Lupinus albus*) and narrow-leafed (*L. angustifolius*) lupins. *Aquaculture,* **426–427**, 296-303.

Sardar P, Randhawa HS, Abid M, Prabhakar SK (2007) Effect of dietary microbial phytase supplementation on growth performance, nutrient utilization, body compositions and haemato-biochemical profiles of *Cyprinus carpio* (L.) fingerlings fed soyprotein-based diet. *Aquac. Nutr.*, **13**, 444-456.

Sarker MSA, Satoh S, Kiron V (2007) Inclusion of citric acid and/or amino acid-chelated trace elements in alternate plant protein source diets affects growth and excretion of nitrogen and phosphorus in red sea bream *Pagrus major*. *Aquaculture,* **262**, 436-443.

Sarker PK, Bureau DP, Hua K, Drew MD, Forster I, Were K, Hicks B, Vandenberg GW (2013) Sustainability issues related to feeding salmonids: a Canadian perspective. *Rev. Aquac.*, **5**, 199-219.

Sathe SK, Reddy NR (2002) Introduction. In: *Food Phytates* (ed. by Reddy NR, Sathe SK). CRC Press, Florida, USA, pp. 280.

Satoh S (2007) Minerals. In: *Dietary supplements for the health and quality of cultured fish* (ed. by Nakagawa H, Sato M, Gatlin III DM). CABI, Wallingford, UK ; Cambridge, MA, pp. 244.

Satoh S, Poe WE, Wilson RP (1989) Effect of supplemental phytate and/or tricalcium phosphate on weight gain, feed efficiency and zinc content in vertebrae of channel catfish. *Aquaculture,* **80**, 155-161.

Schrauzer GN (2000) Selenomethionine: A review of its nutritional significance, metabolism and toxicity. *J. Nutr.*, **130**, 1653-1656.

Schuller D (2010) Better Yeast for Better Wine - Genetic Improvement of *Saccharomyces Cerevisiae* Wine Strains. In: *Progress in Mycology* (ed. by Rai M, Kövics G). Springer Netherlands, pp. 1-49.

Schwarz K, Foltz CM (1957) Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.*, **79**, 3292-3293.

Selle PH, Cowieson AJ, Cowieson NP, Ravindran V (2012) Protein–phytate interactions in pig and poultry nutrition: a reappraisal. *Nutr. Res. Rev.,* **25**, 1-17.

Selle PH, Walker AR, Bryden WL (2003) Total and phytate-phosphorus contents and phytase activity of Australian-sourced feed ingredients for pigs and poultry. *Aust. J. Exp. Agric.,* **43**, 475-479.

Sevgili H, Kurtoğlu A, Oikawa M, Aksoy A, Kocakaya S, Öztürk E, Uysal R, Gündüz Oruç H (2015) A combination of corn gluten and soybean meal substitute for fishmeal in diets of turbot (*Scophthalmus maximus* Linnaeus 1758) in brackish water. *J. Appl. Ichthyol.,* **31**, 355-361.

Shahsavani D, Mohri M, Gholipour Kanani H (2008) Determination of normal values of some blood serum enzymes in *Acipenser stellatus* Pallas. *Fish Physiol. Biochem.,* **36**, 39-43.

Shan Z, Li H, Bao X, He C, Yu H, Liu W, Hou L, Wang J, Zhu D, Sui L, Zhu B, Li Y (2011) A selenium-dependent glutathione peroxidase in the Japanese scallop, Mizuhopecten yessoensis: cDNA cloning, promoter sequence analysis and mRNA expression. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.,* **159**, 1-9.

Shapawi R, Ng W-K, Mustafa S (2007) Replacement of fish meal with poultry by-product meal in diets formulated for the humpback grouper, *Cromileptes altivelis*. *Aquaculture,* **273**, 118-126.

Sharawy Z, Goda AMAS, Hassaan MS (2016) Partial or total replacement of fish meal by solid state fermented soybean meal with *Saccharomyces cerevisiae* in diets for Indian prawn shrimp, *Fenneropenaeus indicus*, Postlarvae. *Anim. Feed Sci. Tech.,* **212**, 90-99.

Shepherd CJ, Jackson AJ (2013) Global fishmeal and fish-oil supply: inputs, outputs and markets. *J. Fish Biol.,* **83**, 1046-1066.

Shi C, He J, Wang J, Yu J, Yu B, Mao X, Zheng P, Huang Z, Chen D (2015) Effects of *Aspergillus niger* fermented rapeseed meal on nutrient digestibility, growth performance and serum parameters in growing pigs. *Anim. Sci. J.*, n/a-n/a.

Shi X, Li D, Zhuang P, Nie F, Long L (2006) Comparative blood biochemistry of Amur sturgeon, *Acipenser schrenckii*, and Chinese surgeon, *Acipenser sinensis*. *Fish Physiol. Biochem.,* **32**, 63-66.

Shimeno S, Mima T, Yamamoto O, Ando Y (1993) Effects of fermented defatted soybean meal in diet on the growth, feed conversion, and body composition of juvenile yellowtail. *Nippon Suisan Gakk.,* **59**, 1883-1888.

Shiu Y-L, Hsieh S-L, Guei W-C, Tsai Y-T, Chiu C-H, Liu C-H (2015a) Using *Bacillus subtilis* E20-fermented soybean meal as replacement for fish meal in the diet of orange-spotted grouper (*Epinephelus coioides*, Hamilton). *Aquac. Res.,* **46**, 1403-1416.

Shiu Y-L, Wong S-L, Guei W-C, Shin Y-C, Liu C-H (2015b) Increase in the plant protein ratio in the diet of white shrimp, *Litopenaeus vannamei* (Boone), using *Bacillus subtilis* E20-fermented soybean meal as a replacement. *Aquac. Res.*, **46**, 382-394.

Sies H (1985) Oxidative stress: Introductory remarks. In: *Oxidative Stress* (ed by Sies H). Academic Press, Amsterdan, pp. 1-7.

Silkin YA, Silkina EN (2005) Effect of hypoxia on physiological-biochemical blood parameters in some marine fish. *J. Evol. Biochem. Physiol.*, **41**, 527-532.

Silva-Carrillo Y, Hernández C, Hardy RW, González-Rodríguez B, Castillo-Vargasmachuca S (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*, **364–365**, 180-185.

Silvestre F, Linares-Casenave J, Doroshov SI, Kültz D (2010) A proteomic analysis of green and white sturgeon larvae exposed to heat stress and selenium. *Sci. Total Environ.*, **408**, 3176-3188.

Small BC, Bilodeau AL (2005) Effects of cortisol and stress on channel catfish (*Ictalurus punctatus*) pathogen susceptibility and lysozyme activity following exposure to *Edwardsiella ictaluri*. *Gen. Comp. Endocrinol.*, **142**, 256-262.

Smith DM, Tabrett SJ, Glencross BD, Irvin SJ, Barclay MC (2007a) Digestibility of lupin kernel meals in feeds for the black tiger shrimp, *Penaeus monodon*. *Aquaculture*, **264**, 353-362.

Smith DM, Tabrett SJ, Irvin SJ, Wakeling J, Glencross BD, Harris D (2007b) Response of the black tiger shrimp, *Penaeus monodon* to feed containing the lupin alkaloid, gramine. *Aquaculture*, **272**, 556-563.

Sokrab AM, Mohamed Ahmed IA, Babiker EE (2014) Effect of fermentation on antinutrients, and total and extractable minerals of high and low phytate corn genotypes. *J. Food Sci. Technol.*, **51**, 2608-2615.

Soltanzadeh S, Esmaeili Fereidouni A, Ouraji H, Khalili KJ (2015) Growth performance, body composition, hematological, and serum biochemical responses of beluga (*Huso huso*) juveniles to different dietary inclusion levels of faba bean (*Vicia faba*) meal. *Aquac. Int.*, **24**, 395-413.

Song Z, Li H, Wang J, Li P, Sun Y, Zhang L (2014) Effects of fishmeal replacement with soy protein hydrolysates on growth performance, blood biochemistry, gastrointestinal digestion and muscle composition of juvenile starry flounder (*Platichthys stellatus*). *Aquaculture*, **426–427**, 96-104.

Sorensen EB, Cumbie P, Bauer T, Bell J, Harlan C (1984) Histopathological, hematological, condition-factor, and organ weight changes associated with selenium accumulation in fish from Belews Lake, North Carolina. *Arch. Environ. Contam. Toxicol.*, **13**, 153-162.

Stone D (2012) Developing aquafeeds to enhance productivity and reduce operating costs. The South Australian Research and Development Institute (SARDI), Australia.

Storebakken T, Shearer KD, Roem AJ (1998) Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. *Aquaculture,* **161**, 365-379.

Suarez JA, Tudela C, Davis D, Daugherty Z, Taynor M, Glass L, Hoenig R, Buentello A, Benetti DD (2013) Replacement of fish meal by a novel non-GM variety of soybean meal in cobia, *Rachycentron canadum*: Ingredient nutrient digestibility and growth performance. *Aquaculture,* **416–417**, 328-333.

Sudaryono, Tsvetnenko, Evans (1999) Evaluation of potential of lupin meal as an alternative to fish meal in juvenile *Penaeus monodon* diets. *Aquac. Nutr.*, **5**, 277-285.

Sun H, Tang J-w, Yao X-h, Wu Y-f, Wang X, Liu Y (2015a) Effects of replacement of fish meal with fermented cottonseed meal on growth performance, body composition and haemolymph indexes of Pacific white shrimp, *Litopenaeus vannamei* Boone, 1931. *Aquac. Res.*, 1-10.

Sun H, Tang J-w, Yao X-h, Wu Y-f, Wang X, Liu Y, Lou B (2015b) Partial substitution of fish meal with fermented cottonseed meal in juvenile black sea bream (*Acanthopagrus schlegelii*) diets. *Aquaculture,* **446**, 30-36.

Sunde RA (2006) Selenium. In: *Present knowledge in nutrition* (ed. by Bowman B, Russell R). International Life Sciences Institute, Washington DC, pp. 480-497.

Sveier H, Lied E (1998) The effect of feeding regime on growth, feed utilisation and weight dispersion in large Atlantic salmon (*Salmo salar*) reared in seawater. *Aquaculture,* **165**, 333-345.

Sweetman JW, Torrecillas S, Dimitroglou A, Rider S, Davies SJ, Izquierdo MS (2010) Enhancing the natural defences and barrier protection of aquaculture species. *Aquacult. Res.,* **41**, 345-355.

Swick RA (2002) Soybean meal quality: assessing the characteristics of a major aquatic feed ingredient. *Global Aquaculture Advocate,* **5**, 46-49.

Tabrett S, Blyth D, Bourne N, Glencross BD (2012) Digestibility of *Lupinus albus* lupin meals in barramundi (*Lates calcarifer*). *Aquaculture,* **364-365**, 1-5.

Tacchi L, Lowrey L, Musharrafieh R, Crossey K, Larragoite ET, Salinas I (2015) Effects of transportation stress and addition of salt to transport water on the skin mucosal homeostasis of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **435**, 120-127.

Tacon AGJ, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, **285**, 146-158.

Tacon AGJ, Metian M (2009) Fishing for aquaculture: non-food use of small pelagic forage fish—a global perspective. *Rev. Fish. Sci.*, **17**, 305-317.

Tacon AGJ, Metian M (2015) Feed matters: satisfying the feed demand of aquaculture. *Rev. Fish. Sci. Aquac.*, **23**, 1-10.

Takagi S, Shimeno S, Hosokawa H, Ukawa M (2000) Replacement of fish meal by combined inclusion of alternative protein sources in a diet for yearling red sea bream, *Pagrus major*. *Suisanzoshoku*, **48**, 545-552.

Takahashi K, Cohen H (1986) Selenium-dependent glutathione peroxidase protein and activity: immunological investigations on cellular and plasma enzymes. *Blood*, **68**, 640-645.

Tanner JE, Fernandes M (2010) Environmental effects of yellowtail kingfish aquaculture in South Australia. *Aquac. Environ. Interact.*, **1**, 155-165.

Tantikitti C, Sangpong W, Chiavareesajja S (2005) Effects of defatted soybean protein levels on growth performance and nitrogen and phosphorus excretion in Asian seabass (*Lates calcarifer*). *Aquaculture*, **248**, 41-50.

Tashjian DH, Teh SJ, Sogomonyan A, Hung SSO (2006) Bioaccumulation and chronic toxicity of dietary l-selenomethionine in juvenile white sturgeon (*Acipenser transmontanus*). *Aquat. Toxicol.*, **79**, 401-409.

Teves JFC, Ragaza JA (2016) The quest for indigenous aquafeed ingredients: a review. *Rev. Aquac.*, **8**, 154-171.

Thiry C, Ruttens A, De Temmerman L, Schneider Y-J, Pussemier L (2012) Current knowledge in species-related bioavailability of selenium in food. *Food Chem.*, **130**, 767-784.

Tibaldi E, Hakim Y, Uni Z, Tulli F, de Francesco M, Luzzana U, Harpaz S (2006) Effects of the partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **261**, 182-193.

Tomás-Vidal A, Martínez-Llorens S, Jambrina C, de Saja González R, Jover Cerdá M (2011) Effects of dietary soybean meal on growth, nutritive efficiency and body composition of cultured tench (*Tinca tinca*). *J. Appl. Ichthyol.*, **27**, 892-896.

TomÁS A, De La GÁNdara F, GarcÍA-Gomez A, PÉRez L, Jover M (2005) Utilization of soybean meal as an alternative protein source in the Mediterranean yellowtail, *Seriola dumerili*. *Aquac. Nutr.*, **11**, 333-340.

Torstensen BE, Espe M, Sanden M, Stubhaug I, Waagbø R, Hemre GI, Fontanillas R, Nordgarden U, Hevrøy EM, Olsvik P, Berntssen MHG (2008) Novel production of Atlantic salmon (*Salmo salar*) protein based on combined replacement of fish meal and fish oil with plant meal and vegetable oil blends. *Aquaculture,* **285**, 193-200.

Troell M, Kautsky N, Beveridge M, Henriksson P, Primavera J, Rönnbäck P, Folke C (2013) Aquaculture. In: *Encyclopedia of Biodiversity (Second Edition)* (ed by Levin SA). Academic Press, Waltham, pp. 189-201.

Tudor KW, Jones MA, Hughes SR, Holt JP, Wiegand BR (2013) Effect of fermentation with *Saccharomyces cerevisiae* strain PJ69-4 on the phytic acid, raffinose, and stachyose contents of soybean meal. *Prof. Anim. Sci.,* **29**, 529-534.

Turnbull JF (2012) 4 - Stress and resistance to infectious diseases in fish. In: *Infectious Disease in Aquaculture* (ed by Austin B). Woodhead Publishing, pp. 111-125.

Tveterås S, Tveterås R (2010) The Global Competition for Wild Fish Resources between Livestock and Aquaculture. *J. Agric. Econ.,* **61**, 381-397.

US-EPA (2004) Draft Aquatic Life Water Quality Criteria for Selenium-2004 US EPA Office of Water, Washington DC.

Usmani N, Jafri AK (2002) Influence of dietary phytic acid on the growth, conversion efficiency, and carcass composition of mrigal *Cirrhinus mrigala* (Hamilton) fry. *J. World Aquacult. Soc.,* **33**, 199-204.

Van Vo B, Phan DP, Nguyen HQ, Fotedar R (2015) Optimised fermented lupin (*Lupinus angustifolius*) inclusion in barramundi *(Lates calcarifer*) juveniles diets. *Aquaculture,* **444**, 62-69.

Varsamos S, Flik G, Pepin JF, Bonga SEW, Breuil G (2006) Husbandry stress during early life stages affects the stress response and health status of juvenile sea bass, *Dicentrarchus labrax*. *Fish Shellfish Immunol.,* **20**, 83-96.

Venou B, Alexis MN, Fountoulaki E, Haralabous J (2006) Effects of extrusion and inclusion level of soybean meal on diet digestibility, performance and nutrient utilization of gilthead sea bream (*Sparus aurata*). *Aquaculture,* **261**, 343-356.

Vidal D, Bay SM, Schlenk D (2005) Effects of dietary selenomethionine on larval rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol.,* **49**, 71-75.

Vielma J, Mäkinen T, Ekholm P, Koskela J (2000) Influence of dietary soy and phytase levels on performance and body composition of large rainbow trout (*Oncorhynchus mykiss*) and algal availability of phosphorus load. *Aquaculture,* **183**, 349-362.

Villasante S, Rodríguez-González D, Antelo M, Rivero-Rodríguez S, Lebrancón-Nieto J (2013) Why are prices in wild catch and aquaculture industries so different? *Ambio,* **42**, 937-950.

Vinagre C, Madeira D, Narciso L, Cabral HN, Diniz M (2012) Effect of temperature on oxidative stress in fish: Lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. *Ecol. Indic.,* **23**, 274-279.

Viyakarn V, Watanabe T, Aoki H, Tsuda H, Sakamoto H, Okamoto N, Iso N, Satoh S, Takeuchi T (1992) Use of soybean meal as a substitute for fish meal in a newly developed soft-dry pellet for yellowtail. *Nippon Suisan Gakk.,* **58,,** 1991-2000.

von Danwitz A, van Bussel CGJ, Klatt SF, Schulz C (2016) Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilisation in turbot (*Psetta maxima* L.). *Aquaculture,* **450**, 405-411.

Wang C, Lovell RT (1997) Organic selenium sources, selenomethionine and selenoyeast, have higher bioavailability than an inorganic selenium source, sodium selenite, in diets for channel catfish (*Ictalurus punctatus*). *Aquaculture*, **152**, 223-234.

Wang KY, Peng CZ, Huang JL, Huang YD, Jin MC, Geng Y (2013) The pathology of selenium deficiency in *Cyprinus carpio* L. *J. Fish Dis.*, **36**, 609-615.

Wang W-N, Wang A-L, Zhang Y-J (2006a) Effect of dietary higher level of selenium and nitrite concentration on the cellular defense response of *Penaeus vannamei*. *Aquaculture,* **256**, 558-563.

Wang W, Mai K, Zhang W, Xu W, Ai Q, Liufu Z, Li H (2012) Dietary selenium requirement and its toxicity in juvenile abalone *Haliotis discus hannai* Ino. *Aquaculture*, **330–333**, 42-46.

Wang X-F, Li X-Q, Leng X-J, Shan L-L, Zhao J-X, Wang Y-T (2014) Effects of dietary cottonseed meal level on the growth, hematological indices, liver and gonad histology of juvenile common carp (*Cyprinus carpio*). *Aquaculture,* **428–429**, 79-87.

Wang Y-B, Xu B-H (2008) Effect of different selenium source (sodium selenite and selenium yeast) on broiler chickens. *Anim. Feed Sci. Tech.,* **144**, 306-314.

Wang Y, Han J, Li W, Xu Z (2007) Effect of different selenium source on growth performances, glutathione peroxidase activities, muscle composition and selenium concentration of allogynogenetic crucian carp (*Carassius auratus gibelio*). *Anim. Feed Sci. Tech.*, **134**, 243-251.

Wang Y, Kong L-J, Li C, Bureau DP (2006b) Effect of replacing fish meal with soybean meal on growth, feed utilization and carcass composition of cuneate drum (*Nibea miichthioides*). *Aquaculture*, **261**, 1307-1313.

Wang Y, Ma XZ, Wang F, Wu YB, Qin JG, Li P (2016a) Supplementations of poultry by-product meal and selenium yeast increase fish meal replacement by soybean meal in golden pompano (*Trachinotus ovatus*) diet. *Aquac. Res.*, **Article in press**, 1-11.

Wang Y, Yu S, Wang Y, Che J, Zhao L, Bu X, Yang Y (2016b) Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion of juvenile *Pseudobagrus ussuriensis*. *Aquac. Res.*, **47**, 3145-3155.

Watanabe T (2002) Strategies for further development of aquatic feeds. *Fish. Sci.*, **68**, 242-252.

Watanabe T, Kiron V, Satoh S (1997) Trace minerals in fish nutrition. *Aquaculture*, **151**, 185-207.

Watanabe T, Viyakarn V, Kimura H, Ogawa K, Okamoto N, Iso N (1992) Utilization of soybean meal as a protein source in a newly developed soft-dry pellet for yellowtail. *Nippon Suisan Gakk.*, **58**, 1761-1773.

Webster CD, Lim CE (2002) Introduction to fish nutrition. In: *Nutrient Requirements and Feeding of Finfish for Aquaculture* (ed. by Webster CD, Lim CE). CABI Publishing, Oxon, pp. 1-27.

Wedemeyer G (1997) Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In: *Fish stress and health in aquaculture* (ed. by Iwama G, Pickering A, Sumpter J, Schreck C). Cambridge University Press, Cambridge, pp. 35-72.

Wedemeyer GA (1996) Basic Physiological Functions. In: *Physiology of Fish in Intensive Culture Systems*. Springer US, pp. 10-59.

Wedemeyer GA, Gould RW, Yasutake WT (1983) Some potentials and limits of the leucocrit test as a fish health assessment method. *J. Fish Biol.*, **23**, 711-716.

Welker T, Barrows F, Overturf K, Gaylord G, Sealey W (2015) Optimizing zinc supplementation levels of rainbow trout (*Oncorhynchus mykiss*) fed practical type fishmeal- and plant-based diets. *Aquac. Nutr.*, n/a-n/a.

Williams KC, Barlow CG, Rodgers L, Agcopra C (2006) Dietary composition manipulation to enhance the performance of juvenile barramundi (*Lates calcarifer* Bloch) reared in cool water. *Aquac. Res.*, **37**, 914-927.

Williams KC, Barlow CG, Rodgers L, Hockings I, Agcopra C, Ruscoe I (2003a) Asian seabass Lates calcarifer perform well when fed pelleted diets high in protein and lipid. *Aquaculture*, **225**, 191-206.

Williams KC, Barlow CG, Rodgers LJ, Ruscoe I (2003b) Potential of meat meal to replace fish meal in extruded dry diets for barramundi, *Lates calcarifer* (Bloch). I. Growth performance. *Aquac. Res.*, **34**, 23-32.

Winner RW, Whitford TC (1987) The interactive effects of a cadmium stress, a selenium deficiency and water temperature on the survival and reproduction of *Daphnia magna* Straus. *Aquat. Toxicol.,* **10**, 217-224.

Winston GW (1991) Oxidants and antioxidants in aquatic animals. *Comp. Biochem. Physiol. C: Pharmacol.,* **100**, 173-176.

Wise DJ, Tomasso JR, Gatlin DM, Bai SC, Blazer VS (1993) Effects of dietary selenium and vitamin E on red blood cell peroxidation, glutathione peroxidase activity, and macrophage superoxide anion production in channel catfish. *J. Aquat. Anim. Health,* **5**, 177-182.

Yajima D, Motani H, Hayakawa M, Sato Y, Sato K, Iwase H (2009) The relationship between cell membrane damage and lipid peroxidation under the condition of hypoxia-reoxygenation: analysis of the mechanism using antioxidants and electron transport inhibitors. *Cell Biochem. Funct.,* **27**, 338-343.

Yamamoto T, Iwashita Y, Matsunari H, Sugita T, Furuita H, Akimoto A, Okamatsu K, Suzuki N (2010) Influence of fermentation conditions for soybean meal in a non-fish meal diet on the growth performance and physiological condition of rainbow trout *Oncorhynchus mykiss*. *Aquaculture,* **309**, 173-180.

Yamamoto T, Matsunari H, Sugita T, Furuita H, Masumoto T, Iwashita Y, Amano S, Suzuki N (2012) Optimization of the supplemental essential amino acids to a fish meal-free diet based on fermented soybean meal for rainbow trout *Oncorhynchus mykiss*. *Fish. Sci.,* **78**, 359-366.

Yamashita M, Yamashita Y (2015) Selenoneine in Marine Organisms. In: *Springer Handbook of Marine Biotechnology* (ed by Kim S-K). Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1059-1069.

Yamashita Y, Yabu T, Yamashita M (2010) Discovery of the strong antioxidant selenoneine in tuna and selenium redox metabolism. *World J. Biol. Chem.,* **1**, 144-150.

Yigit M, Erdem M, Koshio S, Ergun S, Turker A, Karaali B (2006) Substituting fish meal with poultry by-product meal in diets for black Sea turbot *Psetta maeotica*. *Aquac. Nutr.,* **12**, 340-347.

Yuan Y-c, Gong S-y, Yang H-j, Lin Y-c, Yu D-h, Luo Z (2010) Apparent digestibility of selected feed ingredients for Chinese sucker, Myxocyprinus asiaticus. *Aquaculture,* **306**, 238-243.

Yuan YC, Lin YC, Yang HJ, Gong Y, Gong SY, Yu DH (2013) Evaluation of fermented soybean meal in the practical diets for juvenile Chinese sucker, *Myxocyprinus asiaticus*. *Aquac. Nutr.,* **19**, 74-83.

Yue GH, Zhu ZY, Lo LC, Wang CM, Lin G, Feng F, Pang HY, Li J, Gong P, Liu HM, Tan J, Chou R, Lim H, Orban L (2009) Genetic variation and population structure of Asian seabass (*Lates calcarifer*) in the Asia-Pacific region. *Aquaculture,* **293**, 22-28.

Yue Y-R, Zhou Q-C (2008) Effect of replacing soybean meal with cottonseed meal on growth, feed utilization, and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. *Aquaculture*, **284**, 185-189.

Zhang Y, Øverland M, Sørensen M, Penn M, Mydland LT, Shearer KD, Storebakken T (2012a) Optimal inclusion of lupin and pea protein concentrates in extruded diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture,* **344-349**, 100-113.

Zhang Y, Øverland M, Xie S, Dong Z, Lv Z, Xu J, Storebakken T (2012b) Mixtures of lupin and pea protein concentrates can efficiently replace high-quality fish meal in extruded diets for juvenile black sea bream (*Acanthopagrus schlegeli*). *Aquaculture*, **354-355**, 68-74.

Zhang YQ, Wu YB, Jiang DL, Qin JG, Wang Y (2014a) Gamma-irradiated soybean meal replaced more fish meal in the diets of Japanese seabass (*Lateolabrax japonicus*). *Anim. Feed Sci. Tech.*

Zhang YQ, Wu YB, Jiang DL, Qin JG, Wang Y (2014b) Gamma-irradiated soybean meal replaced more fish meal in the diets of Japanese seabass (*Lateolabrax japonicus*). *Anim. Feed Sci. Tech.*, **197**, 155-163.

Zhou F, Song W, Shao Q, Peng X, Xiao J, Hua Y, Owari BN, Zhang T, Ng W-K (2011) Partial replacement of fish meal by fermented soybean meal in diets for black sea bream, *Acanthopagrus schlegelii*, juveniles. *J. World Aquacult. Soc.*, **42**, 184-197.

Zhou Q-C, Yue Y-R (2012) Apparent digestibility coefficients of selected feed ingredients for juvenile hybrid tilapia, *Oreochromis niloticus*×*Oreochromis aureus*. *Aquac. Res.*, **43**, 806-814.

Zhou QC, Mai KS, Tan BP, Liu YJ (2005) Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). *Aquac. Nutr.*, **11**, 175-182.

Zhou X, Wang Y, Gu Q, Li W (2009) Effects of different dietary selenium sources (selenium nanoparticle and selenomethionine) on growth performance, muscle composition and glutathione peroxidase enzyme activity of crucian carp (*Carassius auratus gibelio*). *Aquaculture,* **291**, 78-81.

Zhu Y, Chen Y, Liu Y, Yang H, Liang G, Tian L (2012) Effect of dietary selenium level on growth performance, body composition and hepatic glutathione peroxidase activities of largemouth bass *Micropterus salmoide*. *Aquac. Res.*, **43**, 1660-1668.

Zhu Y, Ding Q, Chan J, Chen P, Wang C (2015) The effects of concurrent supplementation of dietary phytase, citric acid and vitamin D3 on growth and mineral utilization in juvenile yellow catfish *Pelteobagrus fulvidraco*. *Aquaculture*, **436**, 143-150.

Zhuo L-C, Liu K, Lin Y-H (2014) Apparent digestibility of soybean meal and *Lactobacillus* spp. fermented soybean meal in diets of grouper, *Epinephelus coioides*. *Aquac. Res.*, 1-4.

APPENDIX 1

List of Publications

1. Ilham I, Fotedar R (2016) Growth, antioxidant capacity and muscle histochemistry of yellowtail kingfish (*Seriola lalandi* Valenciennes 1883): Selenium and temperature interaction. *Anim. Feed Sci. Tech.*, **217**, 76-86.

2. Ilham I, Fotedar R, Suyasa IN (2016) Use of organic selenium supplements in soybean meal-based diets for juvenile yellowtail kingfish (*Seriola lalandi*). *Int. J Food Nutr. Sci.*, **3**, 1-11.

3. Ilham I, Siddik MAB, Fotedar R (2016) Effects of organic selenium supplementation on growth, accumulation, haematology and histopathology of juvenile barramundi (*Lates calcarifer*) fed high soybean meal diets. *Biol. Trace Elem. Res.*, **174**, 436-447.

4. Ilham I, Fotedar R, Munilkumar S (2016) Effects of organic selenium supplementation on growth, glutathione peroxidase activity and histopathology in juvenile barramundi (*Lates calcarifer* Bloch 1970) fed high lupin meal-based diets. *Aquaculture*, **457**, 15-23.

5. Ilham I, Fotedar R (2017) Growth, enzymatic glutathione peroxidase activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented soybean meal and organic selenium. *Fish Physiol. Biochem.*, **43**, 775-790.

6. Ilham I, Fotedar R (2017) Growth, enzymatic glutathione peroxidase (GPx) activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented lupin meal supplemented with organic selenium. *Aquacult. Res*. Accepted for publication on 20 January 2017.

APPENDIX 2

Abstract of Published Papers

1. Ilham I, Fotedar R (2016) Growth, antioxidant capacity and muscle histochemistry of yellowtail kingfish (*Seriola lalandi* Valenciennes 1883): Selenium and temperature interaction. *Anim. Feed Sci. Tech.*, **217**, 76-86.

ARTICLE INFO

Article history:
Received 10 June 2015
Received in revised form 15 April 2016
Accepted 16 April 2016

Keywords:
Selenium
Temperature
Growth
Antioxidant capacity
Muscle histochemistry
Yellowtail kingfish

ABSTRACT

The aim of this study was to investigate the interactive effects of temperature and dietary selenium concentrations on antioxidant capacity, muscle histochemistry and the growth, of juvenile yellowtail kingfish (*Seriola lalandi*). The yellowtail kingfish were exposed to two temperatures (21 °C or 26 °C) and three selenium levels (0.0, 2.0 or 4.0 mg Se kg^{-1} of feed) for 30 days. Final weight and specific growth rate (SGR) were significantly affected by water temperature ($\rho < 0.001$) and dietary Se ($\rho < 0.001$) supplementation, and there were significant differences in the interaction between these two factors. Juvenile yellowtail kingfish fed Se-supplemented diets, attained a higher final weight and SGR than those without Se supplementation at 21 °C, but not at 26 °C. Regardless of the temperature, the red blood cell (RBC) glutathione peroxidase (GPx) activity of yellowtail kingfish fed Se-supplemented diets was significantly higher ($\rho < 0.05$) than with the control diet. However, GPx activity of yellowtail kingfish when fed either 2.0 mg Se kg^{-1} or 4.0 mg Se kg^{-1} showed no significant difference ($\rho > 0.05$). Se concentration in the muscles of juvenile yellowtail kingfish fed Se-supplemented diets was higher than that of the yellowtail kingfish that were fed the control diet. A histopathological test confirmed that 20.3% of fish muscles exhibited lesions, which occurred in the absence of dietary Se. The outcome of the present study helps in understanding the interactive effects of dietary Se concentrations and the temperature in the farming of yellowtail kingfish.

2. Ilham I, Fotedar R, Suyasa IN (2016) Use of organic selenium supplements in soybean meal-based diets for juvenile yellowtail kingfish (*Seriola lalandi*). *Int. J Food Nutr. Sci.*, **3**, 1-11.

Abstract

Sustainable aquaculture, from both ecological and economic perspectives, demands a substantial reduction of the amount of fishmeal used in aqua feed. Instead, plant-derived protein feed stuff, which provide a nutritious diet offer a promising alternative for inclusion in aqua feed. In this study, the effects of organic selenium supplementation in low and high soybean meal-based practical diets for yellowtail kingfish (*Seriola lalandi*) were evaluated over a 60-day feeding experiment. The juvenile yellowtail kingfish (initial weight 5.02 ± 0.04 g $fish^{-1}$) were fed five iso-nitrogenous and iso-caloric diets containing 49% crude protein and 22 MJ kg^{-1} gross energy. The control diet(C) includes 46% fishmeal, whereas the other diets used soybean meal to replace 25% and 75% of the fishmeal protein, both without and with organic selenium supplementation (SBM_{25}, SBM_{25+Se}, SBM_{75}, and SBM_{75+Se}). Fish were fed *ad libitum* two times a day at 09:00 and 15:00 hours. No differences were observed in feed intake among dietary treatment groups ($P > 0.05$). Organic selenium-supplemented diets improved final weight; however, final weight was significantly reduced when fish were fed high soybean meal diets ($P < 0.05$). Organic selenium supplementation had a significant effect on specific growth rate at low soybean meal diets, but did not affect specific growth rate when fish were fed high soybean meal diets. Selenium accumulation in fish fillet was strongly correlated with selenium concentration in the diets. While fish fed the SBM_{25+Se} diets had significantly higher glutathione peroxidase activity than those fed other diets, the lowest glutathione peroxidase activity was found in fish fed SBM_{75} diets. Histologically, selenium-deficient diets induced myopathy and alterations in tissue structure were most prevalent in fish fed the SBM_{75} diet. The findings of this study indicate that, with Se supplementation, soybean meal could supply 25% of the protein in yellowtail kingfish diets.

Received Date: April 04, 2016
Accepted Date: May 10, 2016
Published Date: May 16, 2016

Citation: Ilham, et al. Use of Organic Selenium Supplements in Soybean Meal-Based Diets for Juvenile Yellowtail Kingfish (*Seriola lalandi*). (2016) Int J Food Nutr Sci 3(2): 1- 11.

DOI: 10.15436/2377-0619.16.860

3. Ilham I, Siddik MAB, Fotedar R (2016) Effects of organic selenium supplementation on growth, accumulation, haematology and histopathology of juvenile barramundi (*Lates calcarifer*) fed high soybean meal diets. *Biol. Trace Elem. Res.*, **174**, 436-447.

Received: 15 December 2015 / Accepted: 14 April 2016

Abstract Soybean meal (SBM) has been commonly utilised as a substitute for fishmeal (FM) in the diets of several fish species. However, little is known regarding their effects on trace element availability and thus their importance to fish. The present study employed two feeding trials to evaluate the implications of dietary selenium (Se) on the growth, accumulation, antioxidant, and histopathological responses of juvenile barramundi (*Lates calcarifer*). In the first trial, each of three basal diets containing 0, 15 and 43 % SBM as replacements for 0, 25 and 75 % of FM protein on an isoproteic and isocalorific basis were either supplemented or not supplemented with 2 mg kg^{-1} organic Se (OS). In the second trial, the potential effect of OS supplementation in a high SBM diet was investigated in a feeding trial with five experimental diets: 75 % SBM protein as replacement of FM was supplemented with 2, 3, 4, 5 or 7 mg OS kg^{-1}. Growth was independently influenced by the SBM level and the OS supplementation level but not by their interaction. Glutathione peroxidase (GPx) activity, haematocrit, Se accumulation and muscle tissue integrity were significantly enhanced in fish fed on OS-supplemented diets. Furthermore, when high SBM was included in diets, elevated Se tended to lower the barramundi's performance. These findings suggest that dietary supplementation of OS at 2–3 g kg^{-1} diet is necessary when high plant protein ingredients are incorporated in the diet, in order to maintain better growth and to afford protection against oxidative stress.

Keywords Organic selenium · Soybean meal · Growth · Glutathione peroxidase · Necrosis · Barramundi

Introduction

The use of fishmeal (FM) as the major protein source for aquaculture production has been plagued by both economic and environmental objections. While FM is disputably sustainable [1, 2], increased demand for FM from rapidly expanding aquaculture can be expected to remain high in the long term [3, 4]. Traditionally, carnivorous fishes perform well on diets high in FM, which contain high protein levels and provide a remarkably good source of essential amino acids (AA), essential fatty acids, phospholipids, nucleotides and macro- and micro-elements [5]. Nevertheless, the substitution of FM with lower-cost, widely available plant protein (PP) ingredients such as soybean meal (SBM) would be likely to improve the sustainability of the aquaculture industry greatly. Moreover, aquafeeds represent the largest portion of the production cost, with FM being the most expensive ingredient. Hence, efforts to find economically viable and environmentally friendly feeds never cease, with researchers and industries continually working to expand their sourcing.

4. Ilham I, Fotedar R, Munilkumar S (2016) Effects of organic selenium supplementation on growth, glutathione peroxidase activity and histopathology in juvenile barramundi (*Lates calcarifer* Bloch 1970) fed high lupin meal-based diets. *Aquaculture*, **457**, 15-23.

ARTICLE INFO

Article history:
Received 16 November 2015
Received in revised form 7 January 2016
Accepted 3 February 2016
Available online 4 February 2016

Keywords:
Selenium
Growth
Glutathione peroxidase
Myopathy
Barramundi

ABSTRACT

Very limited information is available on the relationship between dietary selenium (Se) and plant protein (PP) sources in carnivorous marine aquaculture species. Therefore, this study employed a 2 × 3 experimental layout to investigate the effects of lupin meal (LM) protein inclusion levels (0, 25 and 75%) and organic selenium (OS) levels (0 or 2 g kg^{-1}) on the growth, physiology and histopathology of juvenile barramundi (*Lates calcarifer*). The experimental diets (LM_0, LM_{0+OS}, LM_{25}, LM_{25+OS}, LM_{75} and LM_{75+OS}) were formulated on an isonitrogenous (48.8% crude protein) and isocalorific (20.6 MJ kg^{-1} gross energy) basis. In the 60-day feeding experiment, final weight (FW), specific growth rate (SGR) and weight gain (WG) were improved by the supplementation of Se in LM-based diets. Fish fed diets containing Se had higher FW, SGR and WG compared with those fed diets lacking Se supplementation ($P < 0.05$). Both LM inclusion levels and Se supplementation levels affected the apparent digestibility coefficient of protein (ADC-P). Meanwhile, survival and the thermal growth coefficient (TGC) were not significantly different among all dietary treatments. The inclusion of a high LM level resulted in decreased glutathione peroxidase (GPx) activities, but this effect was not observed when Se was supplemented in the diets. Furthermore, there was a linear relationship between muscle Se level and Se concentration of the experimental diets. Se-induced myopathy was observed in skeletal muscles of fish fed LM diets without Se supplementation. In addition, structural alteration was found in the liver; however, the kidney, spleen and intestine were histologically normal. Overall, these results suggest that high LM diets supplemented with organic selenium can enhance growth, physiological and histological performances of juvenile barramundi.

Statement of relevance: While plant-based feed sources such as lupin meal have the potential to reduce the reliance on unsustainable wild fishmeal in aquaculture, such products may reduce the feed availability of selenium, an essential element for aquatic animals. We believe that the findings of this study are relevant to the general field of commercial aquaculture.

5. Ilham I, Fotedar R (2017) Growth, enzymatic glutathione peroxidase activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented soybean meal and organic selenium. *Fish Physiol. Biochem.*, **43**, 775-790.

Received: 30 March 2016 / Accepted: 12 December 2016

Abstract Solvent-extracted soybean meal (SBM) was fermented using baker's yeast *Saccharomyces cerevisae* at 30 °C for 5 days. Four isonitrogenous and isocaloric diets containing 75% SBM protein, either fermented or non-fermented (SBM and FSBM), and supplemented or not with organic Se (OS) (SBM_{OS} and $FSBM_{OS}$), were fed to triplicate groups of juvenile barramundi (*Lates calcarifer*) (initial weight of 5 g) for 75 days. A fishmeal (FM)-based diet formulated for juvenile barramundi was used as a reference diet. The growth of fish was significantly affected by either the interaction of SBM type or by the OS level. In fish fed diets supplemented with OS (SBM_{OS} and $FSBM_{OS}$), final weight (FW), specific growth rate (SGR) and weight gain (WG) were higher in fish fed the fermented SBM ($FSBM_{OS}$) than in those fed the non-fermented SBM (SBM_{OS}). The apparent digestibility coefficient (ADC) of protein was higher in the fish fed the fermented SBM, either supplemented or unsupplemented with OS. However, there were no significant differences in the ADC of dry matter (DM) and lipids among the tested diets and in comparison to the reference diet. The haematocrit and leucocrit of fish fed the $FSBM_{OS}$ diet were lower than those of fish fed the FM diet. Furthermore, glutathione peroxidase (GPx) activity was significantly influenced by OS supplementation in the experimental diets; GPx activity was greater in the fish fed diets supplemented with OS. Creatinine kinase (CK) of all groups of fish was higher than the CK of those fed the reference diet. These results suggest that with a proper nutritional level, OS supplementation may act as an important factor in enzymatic GPx activity and in the haematology and blood biochemistry status of juvenile barramundi fed fermented SBM-based diets, encouraging improvement of the overall growth performance.

Keywords Fermented soybean meal · Organic selenium · Growth · Glutathione peroxidase · Haematology · Biochemistry status · Barramundi

Introduction

Barramundi (*Lates calcarifer*) is one of the economically important marine finfish species in Asian and

6. Ilham I, Fotedar R (2017) Growth, enzymatic glutathione peroxidase (GPx) activity and biochemical status of juvenile barramundi (*Lates calcarifer*) fed dietary fermented lupin meal supplemented with organic selenium. *Aquacult. Res*. Accepted for publication with minor revision required.

Abstract

To investigate the effects of high level of lupin meal (LM) supplemented with organic selenium (OS) on the growth and blood biochemistry of barramundi (*Lates calcarifer*), four isocaloric and isonitrogenous diets were prepared, containing either non-fermented or fermented LM, and either supplemented with 2 mg OS kg^{-1} (LM, LM_{OS}, FLM and FLM_{OS}), or not. A fishmeal (FM)-based diet formulated for juvenile barramundi was used as a control diet. Fish (initial mean weight of 5.88 g) were triplicated and fed the test diets for 75 days. The findings demonstrated that growth performance of fish fed with the FLM and FLM_{OS} diets were similar to fish fed with the FM diet ($P > 0.05$). The antioxidant glutathione peroxidase (GPx) activity, and haemoglobin (Hb) of fish fed with the FLM_{OS} diet were significantly higher than that of FM-fed fish ($P < 0.05$). Plasma alanine aminotransferase (ALT) activity was significantly increased in fish fed with non-fermented diets (LM and LM_{OS}) than in those fed with fermented LM diets (FLM and FLM_{OS}) ($P < 0.05$). However, there were no significant differences in ALT activity among LM_{OS}, FLM, FLM_{OS} and FM diets. There was an interaction between the LM and OS on plasma CK activity; the CK of fish fed with diets supplemented with OS was higher in non-fermented LM diets but lower in fermented LM diets ($P < 0.05$). The present study suggests that fermented LM simultaneously supplemented with OS have an obvious potential to substantially replace 75% FM protein in the diets of barramundi.