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Growth and physiological responses of snapper (*Pagrus auratus*) and cobia (*Rachycentron canadum*) fed various inclusion levels of selenium supplemented lupin meal as fishmeal replacement diets

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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_11.

Signature

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PREAMBLE

The thesis includes six chapters. The brief overviews about the use of dietary selenium (Se) supplementation, fishmeal replacement in fish are presented in chapter 1. This chapter also clarifies the current issues related to Australian snapper *Pagrus auratus* and cobia *Rachycentron canadum* aquaculture. The aim, main objectives and significance of this research also are highlighted in this chapter.

The literature review in chapter 2 reviews the biology, aquaculture and nutritional research in snapper and cobia. The relevant information on Se and its effects on growth and physiology in fish also are described. The chapter also reviews the nutritional values and uses of lupin meal as alternative protein sources to reduce the fishmeal reliance in aqua-feeds.

The material and methodology of this research are presented in chapter 3. This chapter details the preparation of experimental diets, fish rearing methodologies, sample collections, sample analysis and data analysis of five feeding trials which starts with first trial on evaluating the effects of organic selenium in juvenile snapper fed reconstituted commercial and formulated basal diets. Following sections describe the material and methods of the second trial which determined the effects of various levels of organic Se on juvenile cobia fed a commercial feed. Following sections display the methodologies of the third and fourth feeding experiments to evaluate effects of dietary lupin kernel meal as fishmeal replacement on growth and physiological responses of snapper and cobia, respectively. The material and methods of the last trial on determining the effects of organic Se on juvenile cobia fed lupin-based diets are presented in the last section of this chapter.

All experimental results of the five trials are presented in chapter 4

Chapter 5 summaries and discusses all results in this study. The discussion not only follows the order of five trials but tries to amalgamate the discussion into one coherent theme. The evaluations of the results in the current study with previous research on the use of Se supplementation and plant protein inclusion in aqua-feeds also are discussed in this chapter. The interrelationship between dietary Se and protein sources on growth and physiological responses of fish also are presented and discussed.

The main conclusions and recommendations for future research are highlighted in the chapter 6. The last chapter, the chapter 7 includes all the references used in this thesis.

ABSTRACT

The past literature has demonstrated the beneficial effects of dietary mineral supplementation such as zinc and copper on growth and physiological performances in fish fed plant-based diets. However, the relationship between dietary selenium (Se), an essential mineral for growth and physiological functions in aquatic species, with plant-derived ingredients such as lupin meal is very limited and never researched in commercially important carnivorous marine finfish species such as Australian snapper *Pagrus auratus* and cobia *Rachycentron canadum*. A series of five feeding trials were conducted to investigate the nutritional effects of dietary Se and lupin kernel meal on the responses of growth and physiology of snapper and cobia.

In the first experiment, Australian snapper were fed to a reconstituted commercial diet and formulated basal diet with 0 and 0.8 mg/kg Se supplementation to evaluate the effects of dietary supplemental Se on the growth, feed utilisation, body composition and liver histology of juvenile Australian snapper. In the second experiment, five levels of Se extracted from Se-yeast were supplemented to a commercial cobia feed to determine the dietary Se requirement for juvenile cobia. The third and fourth trials evaluated the growth and physiological responses of both snapper and cobia after being fed to various inclusion levels of 0, 105, 210, 315 and 420 g/kg lupin kernel meal as fishmeal replacement which presented in a comparative study. The last experiment investigated effects of dietary Se supplementation on growth and physiological performances of juvenile cobia fed lupin-based diets.

The results indicated that snapper required a lower concentration of dietary protein than the currently used diets derived from other commercial marine finfish and there is no need to provide supplementary Se to the snapper diet, as basic dietary ingredients are capable of providing adequate Se to meet the nutritional requirements of the species. Juvenile cobia fed available commercial cobia feed containing 1.15 mg/kg showed deficient-Se symptoms. The dietary Se concentration required for juvenile cobia fed pelleted formulated feed was 2.32 mg/kg based on the quadratic regression of specific growth rate, whereas dietary Se of 3.14 mg/kg may be threshold level for the species. Cobia showed less susceptibility to dietary lupin than snapper. Snapper displayed negative growth and feed efficiency with increasing inclusion lupin levels. Meanwhile, the dietary inclusion level of 105 g/kg lupin kernel meal did not impair the growth rates and feed efficiency, digestibility and health of cobia. The histopathological lesions were

observed in the liver tissues of both species when being fed to 420 g/kg lupin meal inclusion. The results also indicated that the faster growing species including cobia needs uptake of more nutrients to satisfy their energy demand for metabolism than those in slower growing fish species such as snapper. Cobia fed lupin-based diets with Se supplementation showed improvements in growth, feed utilisation and physiological performances than those fed diets lacking Se supplementation. The results also indicated that cobia fed plant-derived ingredients as lupin kernel meal required dietary Se at a higher concentration than previously investigated for the same species using a purified diet.

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LIST OF ABBREVIATIONS

ADC	Apparent digestibility coefficient
ANOVA	Analysis of variance
ARSS	Aquacultural Research Seed Production and Services Co., Ltd
BCMI	Batavia Coast Maritime Institute
BS	Basal diet
CF	Condition factor
CD	Reconstituted commercial diet
CV	Coefficient of variance
DM	Dry matter
EAA	Essential amino acids
FCR	Feed conversion ratio
GPx	Glutathione peroxidase
Hb	Haemoglobin
HSI	Hepatosomatic index
Ht	Haematocrit
HUFA	Highly unsaturated fatty acids
LKM	Lupin kernel meal
NRC	National Research Council
PUFA	Polyunsaturated fatty acids
RBC	Red blood cell count
SD	Standard deviation
SE	Standard error
Se	Selenium
SE	Standard error
SEM	Standard error of means
SeCys	selenocysteine
SeMet	Selenomethionine
TGC	Thermal growth coefficient
TEAAs	Total essential amino acids
TNEEAs	Total non-essential amino acids
VSI	Viscerasomatic index
WBC	White blood cell count

LIST OF COMMON AND SCIENTIFIC NAMES

Common Name	Scientific Name
African catfish	*Clarias gariepinus*
Atlantic salmon	*Salmo salar*
Australian snapper	*Pagrus auratus*
Black seabream	*Acathopagrus schlegeli*
Channel catfish	*Ictalurus punctatus*
Cobia	*Rachycentron canadum*
Coho salmon	*Oncorhynchus kisutch*
Common carp	*Cyprinus carpio*
Gibel carp (Crucian carp)	*Carassius auratus gibelio*
Gilthead seabream	*Sparus aurata*
Green sturgeon	*Acipenser medirostris*
Green sunfish	*Lepomis cyanellus*
Grouper	*Epinephelus malabaricus*
Hybrid striped bass	*Morone chrysops* × *M. saxatilis*
Mallard duck	*Anas platyrhynchos*
Mrigal	*Cirrhinus mrigala*
Largemouth bass	*Micropterus salmoide*
Loach	*Paramisgurnus dabryanus*
Mangrove red snapper	*Lutjanus argentimaculatus*
Narrow-leafed lupin	*Lupinus angustifolius*
Blue tilapia	*Oreochromis niloticus*
Olive flounder	*Paralichthys olivaceus*
Rainbow trout	*Oncorhynchus mykiss*
Rohu	*Labeo rohita*
Sacramento splittail	*Pogonichthys macrolepidotus*
Striped bass	*Morone saxitilis*
Tiger bass	*Terapon jurbua*
White lupin	*Lupinus albus*
White sturgeon	*Acipenser transmontanus*
Yellowtail kingfish	*Seriola lalandi*
Yellow lupin	*Lupinus luteus*

LIST OF PUBLICATIONS

Journal articles

1. Pham, H. D., Fotedar, R., Nguyen, C. M., & Siddik, M. B. (2016). Feed utilisation efficiency of lupin inclusion in cobia: Role of dietary organic selenium supplementation. *Modern Applied Science*, 10 (10). doi:10.5539/mas.v10n10p180.
2. Pham, H. D., & Fotedar, R. (2017). Do the dietary ingredients of low-protein formulated diet provide a sufficient selenium source in Australian snapper *Pagrus auratus* diet (Bloch & Schneider 1801). *Animal Feed Science and Technology*, *223*. doi.org/10.1016/j.anifeedsci.2016.11.012
3. Pham, H, D., Fotedar, R., & Nguyen, C. M. Growth, feed efficiency and physiological responses of cobia *Rachycentron canadum* fed various inclusion levels of narrow-leafed lupin *Lupinus angustifolius* kernel meal. Submitted in *Marine and Freshwater Behaviour and Physiology*.
4. Pham, H, D., Fotedar, R., Munilkumar, S., & Nguyen, C. M. Biological effects of dietary selenium supplementation in juvenile cobia *Rachycentron canadum* (Linnaeus, 1766) fed a commercial diet. Submitted in *Journal of Applied Ichthyology*.

Conference Abstract

1. Pham, H, D., & Fotedar, R. (2016). Nutritional, haematological and histological responses of Australian snapper *Pagrus auratus* fed various inclusion levels of narrow-leafed lupin kernel meal. Oral presentation in the 3rd International Conference in Fisheries and Aquaculture – 2016. August, 24 – 26, 2016. Negambo, Sri Lanka.

CHAPTER 1. INTRODUCTION

1.1 BACKGROUND

Carnivorous marine fish species generally require high dietary protein to provide adequate amino acids and nitrogen for the synthesis of protein for maintenance and growth (Fraser & Davies, 2009; Tacon & Metian, 2008). Fishmeal rather than terrestrial plant ingredients contains significantly higher protein content with well-balanced essential amino acids, high nutrient digestibility to meet nutritional requirement of the fish (Gatlin *et al.*, 2007; Olsen & Hasan, 2012). The plant protein ingredients are also known to contain relatively high amounts of anti-nutrients such as protease inhibitors, saponins and non-starch polysaccharides (Francis *et al.*, 2001; Gatlin *et al.*, 2007; Kaushik & Hemre, 2008) that are not associated with the natural feeding habits and nutrition profile of carnivorous marine species (Kaushik & Hemre, 2008). However, the rapid development of aquaculture has led to an increase in the demand for fishmeal coupled with its unstable supply (Olsen & Hasan, 2012). Besides, the high price and environmental impacts relating to use of fishmeal are also challenges for aquaculture industry to reduce fishmeal reliance of aqua-feeds (Hardy, 2010; Olsen & Hasan, 2012). A series of studies have been conducted to determine the economically and environmentally alternatives to fishmeal source for the culture of various species (Kader *et al.*, 2010; Luo *et al.*, 2012; Luo *et al.*, 2013; Regost *et al.*, 1999; Watanabe *et al.*, 2001). Even though, some nutritional drawbacks such as reduction of growth and feed efficiency have been reported in some fish fed high dietary plant ingredients, plant-derived feedstuffs have been proven as the main choice for fishmeal replacement in aqua-feeds, due to their availability, cost-effectiveness and acceptable nutritional characteristics (Olsen & Hasan, 2012).

Meals from lupin such as, narrow-leafed lupin *Lupinus angustifolius*, white lupin *Lupinus albus* and yellow lupin *Lupinus luteus* can be used as alternative protein sources to replace protein from fishmeal in fish diets due to their high protein contents, reasonable prices and availabilities (Pereira & Oliva-Teles, 2004; Salini & Adams, 2014). Protein and lipid contents in lupin seeds range from 32 to 38% and 6 to 9%, respectively, while lupin kernel meals contain higher levels of protein (39-52 %) and lipid (9-11%) (Kaushik & Hemre, 2008) and relatively low anti-nutritional factors (ANFs) such as alkaloids, phytic acid, tannin and oligosaccharides are present in narrow-leafed lupin kernel meal than other plant-derived ingredients (Glencross, 2001; Petterson, 2000). Farhangi and Carter (2001) and (Glencross *et al.*, 2004b) incorporated

up to 40% lupin (narrow-leafed and yellow lupins) in rainbow trout *Oncorhynchus mykiss* diets, without any adverse effects in growth performances, while 50 % whole grain white lupin could be included in the diet of this species without reducing growth and feed utilisation (Borquez *et al.*, 2011). Salini and Adams (2014) showed that the dietary inclusion of 20 % narrow-leafed lupin or white lupin had no negative effects on growth and the feed conversion ratio of Atlantic salmon *Salmon salar*, while no significant effects were observed in growth and feed utilisation in gilthead seabream *Sparus aurata* fed a diet with 39 % narrow-leafed lupin seed meal (Pereira & Oliva-Teles, 2004; Robaina *et al.*, 1995). However, the high levels of lupin inclusion in the diet impaired the growth and feed efficiency of rainbow trout (Glencross *et al.*, 2004b), and caused ulcer-like lesions in the stomach of Atlantic salmon (Refstie *et al.*, 2006).

The increased inclusion levels of plant-derived ingredients in aqua-feeds might impact the uptake and digestion of minerals, consequently, changing the mineral needs in fish (Antony Jesu Prabhu *et al.*, 2016; Barrows *et al.*, 2010; Read *et al.*, 2014). This is attributed to the interactions of anti-nutrients presented in plant-based diets and minerals, making these mineral become less availability for fish. The chelation of phytate or tannin with minerals in protein and amino acids has been evidenced in the reduction of nutrient digestibility and growth in fish and other animals (Antony Jesu Prabhu *et al.*, 2016; Ilham *et al.*, 2016a; Kumar *et al.*, 2012; Petterson, 2000). It is recognised that dietary mineral supplementation into plant-based diets to satisfy nutritional requirement can be an alternative approach to enhance beneficial aspects of plant protein in fish, as evidenced in rainbow trout (Barrows *et al.*, 2010; Read *et al.*, 2014), African catfish *Clarias gariepinus* (Abdel-Tawwab *et al.*, 2007) and barramundi *Lates calcarifer* (Ilham *et al.*, 2016a).

Selenium (Se) is an essential trace element for normal growth and physiological functions of animal (Watanabe *et al.*, 1997). However, the deficient and/or excessive dietary Se levels can cause negative effects on growth, survival, peroxidative damage to cells and membranes (Arteel & Sies, 2001; Lin & Shiau, 2005; Liu *et al.*, 2010) and reduced host defence function (Liu *et al.*, 2010; Sweetman *et al.*, 2010; Wang *et al.*, 2013) in fish. Feed ingredients contain varied amounts of Se, with relatively lower Se contents in plant-derived products than fishmeal (Antony Jesu Prabhu *et al.*, 2016; Watanabe *et al.*, 1997). Additionally, the low Se level in lupin meal (18 – 240 µg/kg) in Australia due to the low Se concentration in Australian soils (Petterson, 2000), probably results in the inadequacy of Se in the diets when fishmeal protein is replaced with lupin

meal protein, consequently, impairing the growth and health status. Ilham *et al.* (2016a) showed that the reduction in growth and feed efficiency in barramundi fed high inclusion levels of lupin kernel meal which corresponded with decreasing dietary Se level from 3.11 mg/kg in the fishmeal-based diet to 1.58 mg/kg in lupin-based diet. Due to the lack of Se supplementation, the hybrid striped bass *Morone chrysops* × *M. saxatilis* fed diets containing soybean, or casein resulted in lower growth and glutathione peroxidase (GPx) activity compared to fish fed fishmeal based diet (Cotter, 2006). Whereas, soybean-based diets for rainbow trout fry need to be supplemented with Se to achieve optimum GPx activity (Fontagné-Dicharry *et al.*, 2015). Similarly, Abdel-Tawwab *et al.* (2007) also observed the improved growth performance, feed utilisation and health in African catfish fed plant-based diet supplemented organic Se. Literature also indicated that the fish fed plant-based diets could require nutrient requirements at higher concentrations than those recommended by NRC (2011) to improve growth and feed utilisation (Barrows et al., 2008*;* Barrows et al., 2010*;* Read et al., 2014). Clearly, Se is limiting trace element, and need to be concerned when high plant-derived ingredients are incorporated into fish diets.

The Australian snapper *Pagrus auratus* and cobia *Rachycentron canadum* have become important marine finfish cultured in Asian countries due to their high flesh qualities and market price (Chou *et al.*, 2001; Huang *et al.*, 2007; Nhu *et al.*, 2011; Rahimnejad & Lee, 2013; Zhou *et al.*, 2011). However, the reliance on imported high-cost commercial feeds, difficulties in storage, low feed efficiency and adverse effects on water quality caused by using trash fish are the limiting factors for the expansion of snapper culture in Australia (Booth *et al.*, 2005) and cobia culture in Vietnam (Nhu *et al.*, 2011; Zhou *et al.*, 2011). To develop sustainable culture, some studies have focussed on determining the nutrient requirements of cobia (Chou *et al.*, 2001; Craig *et al.*, 2006; Mai *et al.*, 2009; Zhou *et al.*, 2012; Zhou *et al.*, 2007; Zhou *et al.*, 2006), and replacing fishmeal with availably alternative ingredients for snapper (Booth *et al.*, 2012; Quartararo *et al.*, 1998a) and cobia (Chou *et al.*, 2004; Luo *et al.*, 2012; Luo *et al.*, 2013; Romarheim *et al.*, 2008; Salze *et al.*, 2010; Zhou *et al.*, 2005).

In nature, both snapper and cobia are carnivorous species, with cobia showing relatively faster growth rate than snapper. Therefore, it can be assumed that cobia requires significantly higher feed intake and has higher metabolic rate than snapper to maximise its growth potential. Consequently, snapper and cobia could show different pathways in uptake and digestion of nutrients due to differences in their digestible capacities and

growth rates, as reported in rainbow trout and barramundi (Glencross *et al.*, 2011), rainbow trout and Atlantic salmon (Glencross *et al.*, 2004a; Refstie *et al.*, 2000) and between rainbow trout and snapper (Glencross *et al.*, 2004c). However, there is very limited information on the biological effects of dietary inclusion levels of trace minerals such as Se as well as lupin kernel meal in snapper and cobia. Further, the interactive and/or synergistic roles of organic Se and lupin kernel meal in replacing fishmeal in two distinct carnivorous marine fish is not investigated so far.

1.2 AIM

The study aims to understand the growth performance, feed efficiency and physiological responses of juvenile Australian snapper and cobia fed various dietary levels of selenium in lupin-based diets.

1.3 OBJECTIVES

The aim of the research can be achieved by addressing the following objectives:

1. To evaluate the effects of dietary organic Se supplementation on growth performances, body composition and histological changes of the juvenile snapper.
2. To understand the effects of dietary organic Se supplementation on the growth and physiological responses of the juvenile cobia fed a commercial diet.
3. To compare the growth, feed utilisation, digestibility, haematological and histological performances of snapper and cobia fed various inclusion levels of lupin kernel meal, replacing fishmeal protein.
4. To evaluate the effects of organic selenium supplementation on growth, digestibility, haematological responses of juvenile cobia fed lupin-based diets.

1.4 SIGNIFICANCE

The present study should make significant contributions in improving the marine finfish aquaculture by contribution to the understanding of the effective uses of dietary organic selenium supplementation and lupin protein meal for snapper and cobia aquaculture. The specific significances of the present study are outlined as follows:

- The study will assist in understanding the discrepancies in biological effects of Se in different growth rate marine finfish.

- The research will contribute new findings to understanding the variability in Se requirements in fish due to the inconsistency in the dietary formulation.
- The study can also provide new information on the Se threshold levels for both snapper and cobia that may be applied to diet formulation to prevent the Se toxicity for these species.
- The growth and physiological responses of snapper and cobia fed various inclusion levels of lupin kernel meal in the current study can contribute to understandings the different mechanisms of absorption and digestion of lupin protein meal in two different species.
- The research will contribute to the basic knowledge of cobia immunity and physiology under the influence of dietary organic selenium and alternative protein sources.
- The study will contribute to sustaining the marine finfish aquaculture via reducing the fishmeal reliance by increasing the uses of plant-derived ingredients.

CHAPTER 2. LITERATURE REVIEW

2.1 SELENIUM

2.1.1 Selenium sources and bioavailability

In nature, selenite and selenate are inorganic forms, while organic Se forms comprise selenomethionine, selenium-methylselenomethionine (SeMet), selenocystine and selenocysteine (SeCys) (Watanabe *et al.*, 1997) which result in different pathways on absorption and metabolism in animal (Burk, 1976). Thus, the optimum Se required for fish can vary due to the bioavailability of Se sources. Lin (2014) determined the optimum Se (mg/kg) requirement for grouper *Epinephelus malabaricus* fed dietary selenomethionine and sodium selenite to be 0.98 and 0.90, respectively. Grouper fed organic Se also resulted in relatively higher muscle Se deposition than the fish fed inorganic Se compound. Previous studies have indicated that the organic Se forms showed more bioavailability than inorganic compounds in grouper (Lin, 2014), yellowtail kingfish *Seriola lalandi* (Le & Fotedar, 2014a), gibel carp *Carassius auratus gibelio* (Wang *et al.*, 2007; Zhou *et al.*, 2009), Atlantic salmon (Bell & Cowey, 1989) and channel catfish *Ictalurus punctatus* (Wang & Lovell, 1997). The bioavailability of Se sources in fish is displayed in Table 2.1.1.

2.1.2 Selenium requirement in fish

Fish can absorb Se from water through gills to meet their needs (Hilton *et al.*, 1982; Watanabe *et al.*, 1997). The Se concentration of less than 0.1 µg/L in water enhance the Se uptake through gills and stores as inorganic forms (Watanabe *et al.*, 1997). The minimal dietary Se was quantified for channel catfish to be 0.25 mg/kg with adequate vitamin E and Se level in water less than 2.5 µg/L, whereas this concentration increased to 0.28 mg/kg when Se in rearing water was reduced to 0.4 µg/L (Gatlin & Wilson, 1984). In natural water, Se is present in Se^{0}, Se^{2-}, Se^{4+} and Se^{6+} forms (Liu *et al.*, 2007). The Se concentration in water has strong relationship with Se levels in sediments, where it can rapidly be dissolved in water and transform into selenite which can be absorbed by aquatic animals (Liu *et al.*, 2007; William *et al.*, 2010), while the degradation of aquatic organisms can also redistribute Se forms to sediments through transformation of SeMet, SeCys to Se^{0} and binding to organic matter (Zhang & Moore, 1997).

Dietary Se requirements have been quantified for grouper (Lin, 2014; Lin & Shiau, 2005), gibel carp (Wang *et al.*, 2007; Zhou *et al.*, 2009), black seabream *Acathopagrus*

schlegeli (Lee *et al.*, 2008), cobia (Liu *et al.*, 2010), largemouth *Micropterus salmoide* (Zhu *et al.*, 2012) and yellowtail kingfish (Le & Fotedar, 2013) with varied results, probably due to the differences in Se sources and its bioavailability, protein ingredients, Se concentrations in rearing water as well as different growth rates among different fish species.

Table 2.1.1 Bioavailability of dietary Se forms in fish

Species	Se source	Protein source	Dose Se (mg/kg)	Period (days)	Se bioavailability	References
Atlantic salmon *Salmo salar*	Fishmeal SeMet SeCys Selenite	Fishmeal	1	28	Digestibility of Se in order: SeMet>Selenite>Se Cys>Fishmeal	Bell and Cowey (1989)
Atlantic salmon *Salmo salar*	SeMet Selenite	Fishmeal	1 and 2	56	Higher Se accumulations in muscle and whole body of fish fed Se-Met than selenite	Lorentzen *et al.* (1994)
Channel catfish *Ictalurus punctatus*	SeMet, Se-yeast, Selenite	Casein	0.02, 0.06, 0.20 and 0.40	63	Organic Se displayed higher bioavailability for growth, GPx activity and Se depositions in tissues than selenite	Wang and Lovell (1997)
Crucian carp *Carassius auratus gibelio*	SeMet Selenite	Casein	0.5	30	SeMet resulted in higher muscle Se content and GSH in plasma than selenite	Wang *et al.* (2007)
Crucian carp *Carassius auratus gibelio*	SeMet Se-Nano	Casein	0.5	30	Se-Nano resulted in higher muscle Se content than Se-Met	Zhou *et al.* (2009)
Rainbow trout *Oncorhynchus mykiss*	Se-yeast Selenite	Commercial feed	2, 4 and 8	70	Se-yeast was beneficial in increasing Se accumulation, GSH activity during stress than selenite.	Rider *et al.* (2009)
Grouper *Epinephelus malabaricus*	SeMet Selenite	Casein	0.3, 0.7, 1.0 and 1.5	56	Higher muscle Se accumulation in fish fed SeMet than those fed selenite	Lin (2014)
Yellowtail kingfish *Seriola lalandi*	Se-yeast SeMet SeCys Selenite Fishmeal	Fishmeal	2	42	Se digestibility in order: SeMet, Se-yeast>SeCys, selenite>Fishmeal. SeMet and Se-yeast resulted in higher weight gain, muscle Se and bactericidal activity than selenite	Le and Fotedar (2014a)

Table 2.1.2 Dietary Se requirement in fish

Species	Se source	Protein source	Optimum Se (mg/kg)	References
Rainbow trout *Oncorhynchus mykiss*	Selenite	Casein	0.15 - 0.38	Hilton *et al.* (1980)
Channel catfish *Ictalurus punctatus*	Selenite	Casein	0.25	Gatlin and Wilson (1984)
Black seabream *Acathopagrus schlegeli*	Selenite	Casein	0.21	Lee *et al.* (2008)
Grouper *Epinephelus malabaricus*	Se-Met	Casein	0.70	Lin and Shiau (2005)
Grouper *Epinephelus malabaricus*	Selenite	Casein	0.90	Lin (2014)
Grouper *Epinephelus malabaricus*	Se-Met	Casein	0.98	Lin (2014)
Cobia *Rachycentron canadum*	Se-Met	Casein	0.79 - 0.81	Liu *et al.* (2010)
African catfish *Clarias gariepinus*	Se-yeast	Plant meal	3.67	Abdel-Tawwab *et al.* (2007)
Gibel carp *Carassius auratus gibelio*	Se-Met	Casein	1.18	Han *et al.* (2011)
Hybrid striped bass *Morone chrysops* × *M. saxatilis*	Selenite	Fishmeal	1.81	Cotter *et al.* (2008)
Hybrid striped bass *Morone chrysops* × *M. saxatilis*	Se-yeast	Fishmeal	1.61	Cotter *et al.* (2008)
Largemouth *Micropterus salmoide*	Selenite	Fishmeal	1.6 – 1.85	Zhu *et al.* (2012)
Yellowtail kingfish *Seriola lalandi*	Se-yeast	Fishmeal	4.91 – 15.43	Le and Fotedar (2014b)
Coho salmon *Oncorhynchus kisutch*	Selenite	CD diet	8.6 max	Felton *et al.* (1996)
Yellowtail kingfish *Seriola lalandi*	Se-yeast	CD diet	5.56	Le and Fotedar (2013)

Se requirement is also species dependant, but no research has explained the reasons behind species-specificity. Although, fishmeal-based diets can provide adequate amounts of Se to meet nutritional requirements in some fish (Watanabe *et al.*, 1997), dietary Se supplementation in commercial or low-protein fishmeal diets is necessary to enhance growth, feed utilisation and physiological performances, as in yellowtail kingfish (Le & Fotedar, 2013, 2014b), African catfish (Abdel-Tawwab *et al.*, 2007), largemouth (Zhu *et al.*, 2012) and barramundi (Ilham *et al.*, 2016a). Le and Fotedar (2013) and Liu *et al.* (2010) described higher Se requirements in yellowtail kingfish and cobia due to their

higher growth rates. The higher metabolic rates associated with faster-growing fish require sufficient energy to maximize their growth potential (DeVries & Eastman, 1981), resulting in a need to uptake more nutrients, including Se to meet their nutritional requirements. The dietary Se requirement for fish is displayed in Table 2.1.2.

2.1.3 Selenium deficiency and toxicity

Although, Se is an essential trace element for normal growth and physiological function in fish (Watanabe *et al.*, 1997), but can be harmful at higher dietary levels resulting in growth and feed efficiency reduction (Le & Fotedar, 2014b; Lee *et al.*, 2010), histopathological alterations in digestive tissues such as livers, spleens, kidneys (Le & Fotedar, 2014b; Lee *et al.*, 2008; Lee *et al.*, 2010), reproductive teratogenesis (Lemly, 2002b). Simultaneously, Se-deficiency can cause negative effects on growth and survival, and may lead to peroxidative damage to cells and membranes (Arteel & Sies, 2001; Lin & Shiau, 2005; Liu *et al.*, 2010) and reduced host defence function (Liu *et al.*, 2010; Sweetman *et al.*, 2010; Wang *et al.*, 2013). However, the deficient or toxic threshold of Se in fish considerably varies, depending on protein ingredients, Se sources and different species. The deficiency and toxicity of dietary Se are presented in Table 2.1.3 and Table 2.1.4.

Table 2.1.3 Effects of Se deficiency in fish

Species	Dietary Se (mg/kg)	Fish size (g)	Exposure period (weeks)	Signs of Se deficiency	References
Channel catfish *Ictalurus punctatus*	0.06	4.7	26	Growth and GPx activity depressions, severe myopathy and high mortality	Gatlin *et al.* (1986)
Gibel carp *Carassius auratus gibelio*	0.34	2.74	14	Reduced growth, feed intake and GPx activity	Han *et al.* (2011)
Yellowtail kingfish *Seriola lalandi*	2.31	19.5	10	Reduced growth, feed intake and GPx activity	Le and Fotedar (2014b)
Cobia *Rachycentron canadum*	0.20	6.27	10	High mortality, reduced growth rate, feed efficiency and GPx activity	Liu *et al.* (2010)
Grouper *Epinephelus malabaricus*	0.21	12.2	8	Growth and feed depression, reduced GPx activity	Lin and Shiau (2005)
Grouper *Epinephelus malabaricus*	0.17	24.4	8	Reduced feed efficiency,	Lin (2014)

The erroneous replacement of Se for sulphur during protein synthesis could be a reason for the toxic effects of Se (Janz *et al.*, 2010). In excessive Se supply, the triselenium linkage (Se-Se-Se) or a selenotrisulphide linkage (S-Se-S), instead of disulphide S-S linkages are formed which have key roles for the normal tertiary structure of protein molecules, resulting in the dysfunction of proteins (Maier & Knight, 1994).

Table 2.1.4 Toxic levels of Se in fish

Species	Dietary Se and source (mg/kg)	Fish size (g)	Feeding period (weeks)	Signs of Se toxicity	References
Sacramento splittail *Pogonichthys macrolepidotus*	26.0 Se-yeast	6.8	5 months	Liver alterations, high mortality, reduced growth.	Teh *et al.* (2004)
Sacramento splittail *Pogonichthys macrolepidotus*	6.6 Se-yeast	6.8	9 months	Liver alterations	Teh *et al.* (2004)
White sturgeon *Acipenser transmontanus*	41.7	29.8	8	Reduction in growth and feed intake, histological damage in liver.	Tashjian *et al.* (2006)
Black seabream *Acathopagrus schlegeli*	12.3 Selenite	7.0	15	Reduced growth, feed utilisation. Increased histological damage in tissues	Lee *et al.* (2008)
Olive flounder *Paralichthys olivaceus*	7.38 SeMet	5.0	10	Reduction in growth and survival, histological lesions in liver tissues.	Lee *et al.* (2010)
Loach *Paramisgurnus dabryanus*	0.62 Selenite	6.26	8.5	Liver damages, Reduction in red blood cell, haemoglobin	Hao *et al.* (2014)
Yellowtail kingfish *Seriola lalandi*	20.87 SeMet	19.5	10	Reduced growth and damage in liver and spleen tissues	Le and Fotedar (2014b)
Grouper *Epinephelus malabaricus*	1.52 Selenite	24.4	8	Growth and feed efficiency depression	Lin (2014)
Grouper *Epinephelus malabaricus*	1.49 SeMet	24.4	8	Reduced feed efficiency,	Lin (2014)

However, in the amino acid structure, the terminal methyl group can protect Se in SeMet form (Egerer-Sieber *et al.*, 2006; Mechaly *et al.*, 2000), whereas the selenocysteinyl-tRNA controls the incorporation of SeCys into proteins at the ribosomal level (Stadtman, 1996), consequently, the Se required for structure or function of protein is specifically

incorporated in the polypeptide via the mRNA sequence. Thus, both SeMet and SeCys may not cause the dysfunctional proteins (Janz *et al.*, 2010).

2.1.4 The interaction between Se and other nutrients

The biological effects of interaction between dietary Se and vitamin E on physiological functions of Se have been demonstrated in fish (Jaramillo *et al.*, 2009; Le *et al.*, 2014b; Lin & Shiau, 2009). The effectiveness of Se is through GPx activity, whereas vitamin E is a part of membrane antioxidant, thus the interaction of these nutrients is beneficial in protecting biological membranes against lipid oxidation (Watanabe *et al.*, 1997). The peroxides formation can improve the functions of vitamin E, whereas Se is responsible for peroxide degradation, thus the dietary Se need in fish may vary, depending on the concentration of dietary vitamin E (Watanabe *et al.*, 1997), as reported in grouper, where the dietary Se requirement was reduced from 1.6 to 0.4 mg/kg when dietary vitamin E increased from 50 to 200 mg/kg (Lin & Shiau, 2009).

Besides, the interactions between Se and other elements such as arsenic, sulphur, mercury, cadmium, copper have also been revealed in fish (Dang & Wang, 2011; Watanabe *et al.*, 1997). The mercury bioaccumulation reduced in tiger bass *Terapon jurbua* fed dietary Se supplementation (Dang & Wang, 2011). Lorentzen *et al.* (1998) showed that elevated dietary copper decreased the Se accumulation in the livers of Atlantic salmon that could be attributable to the forming of Se-copper compounds in the intestine, reduction of Se availability and their complexes in the livers.

The interactive effects between Se and plant-derived ingredients have also been reported in some fish species (Abdel-Tawwab *et al.*, 2007; Ilham *et al.*, 2016a; Ilham *et al.*, 2016b). Due to the lower Se concentrations in plants feedstuffs compared to fishmeal (Antony Jesu Prabhu *et al.*, 2016; Watanabe *et al.*, 1997), the increased dietary plant meal in aqua-feeds results in the variation of Se supply in fish, consequently, affecting the growth and physiological performances. Barramundi fed either lupin kernel meal or soybean meal resulted in the growth and feed efficiency reductions, reduced GPx activity as well as histopathological damages in livers, corresponded with decreasing dietary Se level from 3.11 and 3.15 mg/kg in the fishmeal-based diet to 1.58 and 1.53 mg/kg in lupin-based diet and soybean-based diet, respectively (Ilham *et al.*, 2016a; Ilham *et al.*, 2016b). Interestingly, barramundi fed plant-based diet with supplemental Se showed improved growth, physiological and histological performances, as were those in fishmeal diets (Ilham *et al.*, 2016a; Ilham *et al.*, 2016b). Similarly, Abdel-Tawwab *et al.* (2007)

also observed the improved growth performance, feed utilization and health in African catfish fed plant-based diet supplemented organic Se. The hybrid striped bass fed diets containing soybean or casein required Se supplementation to enhance growth and GPx activity (Cotter, 2006), similar to data reported in rainbow trout fry fed soybean-based diets (Fontagné-Dicharry *et al.*, 2015). It seems that the Se fortification in fish fed high inclusion levels of plant-derived ingredients is inevitable to optimise growth, feed efficiency and physiological responses.

2.1.5 Effects of dietary selenium on growth and feed utilization in fish

Due to the narrow margin between deficiency, efficiency and toxicity of Se in fish, the changing in dietary Se supply may rapidly affect the growth and feed utilisation responses (Hodson & Hilton, 1983; Watanabe *et al.*, 1997). Literature reviews have indicated the improvement in growth, feed utilization in fish fed adequate Se diets (Abdel-Tawwab *et al.*, 2007; Le & Fotedar, 2013, 2014a; Lin & Shiau, 2005; Liu *et al.*, 2010). Meanwhile, fish fed deficient Se diets generally result in growth and feed utilisation depression. Cobia fed casein-based diet containing 0.21 mg/kg Se resulted in reducing growth and feed efficiency and high mortality compared to those fed sufficient Se diets (Liu *et al.*, 2010). Similar results were also reported in grouper fed to 0.17 mg/kg Se in casein-based diets (Lin & Shiau, 2005). The weight gain and feed intake were significantly reduced in yellowtail kingfish fed to 3.35 and 2.21 mg/kg Se, respectively (Le & Fotedar, 2013, 2014b).

The excessive dietary Se levels can also negatively impact the growth and feed efficiency in fish. The reduced weight gain and feed intake have been reported in yellowtail kingfish (Le & Fotedar, 2014b), black seabream (Lee *et al.*, 2008), olive flounder *Paralichthys olivaceus* (Lee *et al.*, 2010) and rainbow trout (Hilton *et al.*, 1980) fed to 20.87, 12.3, 7.38 and 13.06 mg/kg Se, respectively. The increased mortality was also observed in olive flounder (Lee *et al.*, 2010) and rainbow trout (Hilton *et al.*, 1980) fed to excessive Se diets.

However, the impacts of dietary Se on growth and feed utilization in fish appear differently, depending on dietary Se sources, exposed period and specific-species. Fish fed dietary Se in organic forms such as SeMet, SeCys and/or Se-yeast resulted in higher growth rate than those fed inorganic Se forms, as reported in juvenile yellowtail kingfish (Le & Fotedar, 2014a) and grouper (Lin, 2014). Olive flounder did not show any differences in growth and feed efficiency after being fed to dietary Se levels for 4 weeks,

however, the growth rate and feed utilization were significantly reduced in fish fed high dietary Se levels after 10 weeks exposure (Lee *et al.*, 2010), as in yellowtail kingfish (Le & Fotedar, 2014b).

Dietary Se also affects the nutrient composition in fish. Olive flounder showed the lipid reduction in the whole-body corresponded with increasing dietary Se levels (Lee *et al.*, 2010). In contradiction, dietary Se had no significant effects on the proximate composition in the muscle tissues of yellowtail kingfish (Le & Fotedar, 2014b) and largemouth bass (Zhu *et al.*, 2012). However, cobia showed increased protein and lipid concentrations in the whole-body, corresponding with the increasing dietary Se levels (Liu *et al.*, 2010). Similarly, in largemouth, where liver lipid were significantly increased as increasing dietary Se levels, which was attributable to the different pathways in lipoprotein uptake and accumulation, hepatic fatty acid synthesis and lipid metabolism in liver tissues impacted by a specific Se mechanism (Zhu *et al.*, 2012).

A series studies have stated the positive relationship between dietary Se and tissue Se deposition in fish (Le & Fotedar, 2014b; Lin & Shiau, 2005; Liu *et al.*, 2010; Wang *et al.*, 2007; Zhu *et al.*, 2012). Tashjian *et al.* (2006) demonstrated the increased Se concentrations with no breakpoints in the kidney, muscle, liver, gill and plasma tissues of white sturgeon *Acipenser transmontanus* fed up to 191.1 mg/kg Se for 8 weeks, similar in black seabream after being fed up to 12.3 mg/kg Se for 15 weeks (Lee *et al.*, 2008). In fish, the Se deposition is affected by dietary Se sources. Lorentzen *et al.* (1994) evaluated the muscle Se concentrations in Atlantic salmon after being fed the fishmeal-based diet supplemented with 1 and 2 mg/kg Se in the form of selenite or SeMet. The muscle Se levels were considerably higher in fish fed dietary SeMet supplementation (1.57 and 2.51 mg/kg) than those fed supplemental selenite (0.43 and 0.57 mg/kg, respectively). Le and Fotedar (2014a) also demonstrated a higher muscle Se accumulations in yellowtail kingfish fed Se-yeast and SeMet than those fed inorganic Se. The reason for this difference is probably due to the different absorption and digestion pathways for Se. In animal, SeMet is metabolized following the methionine pathways, where it is readily assimilated into proteins and then accumulated in liver and muscle tissues (Figure 2.1.1) (Terry & Diamond, 2012; Yeh *et al.*, 1997), wherein selenite is converted to selenide before binding with albumin or hemoglobin and transported to liver for further processes (Haratake *et al.*, 2008).

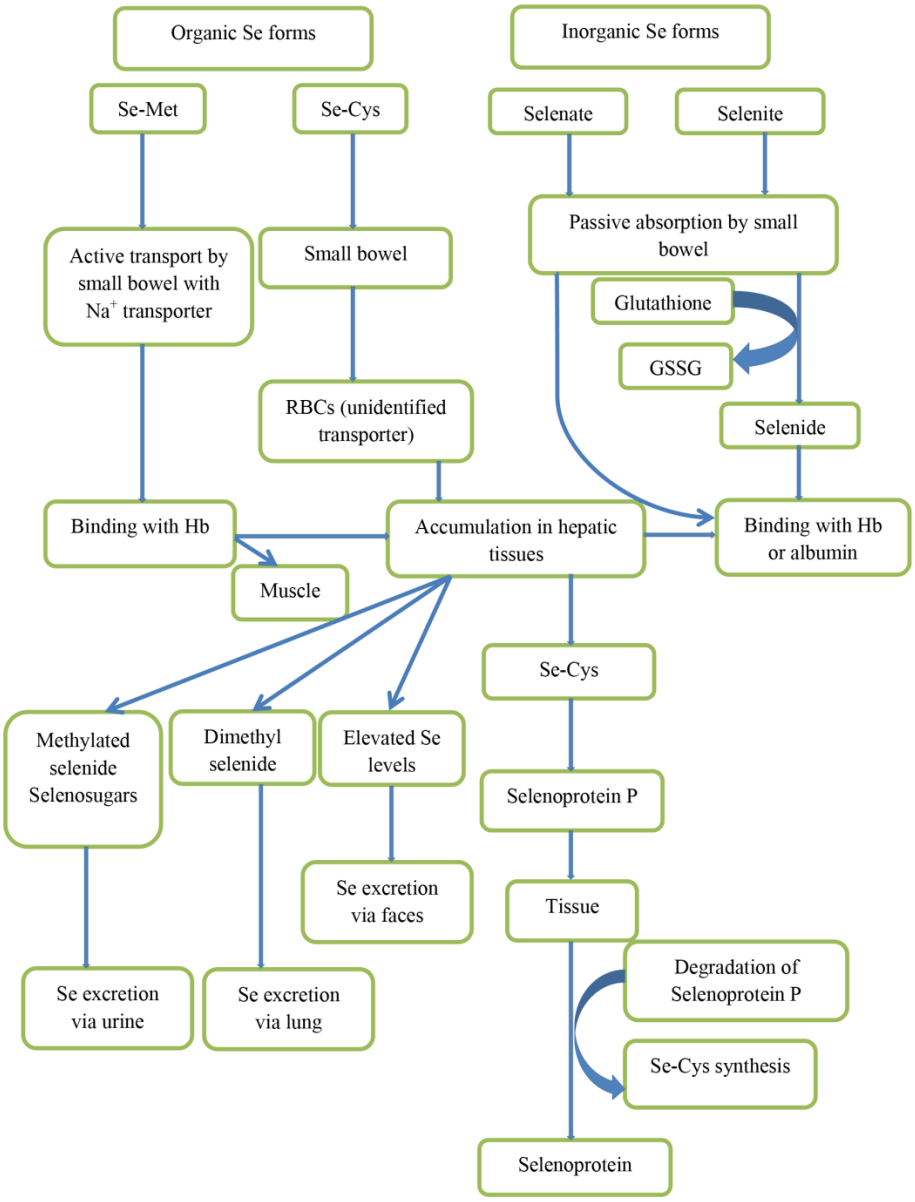


Figure 2.1.1 Diagram of selenium metabolism (Bügel *et al.*, 2008; Burk & Hill, 2009; Suzuki *et al.*, 2005; Terry & Diamond, 2012)

Compared to other tissues, the accumulated Se level in the liver tissue is relatively higher, which probably relates to its important roles in controlling Se excretion and transportation in body (Terry & Diamond, 2012). Thus, the Se level in the liver can be used as bio-marker of dietary Se exposure.

2.1.6 Effects of dietary selenium on physiological responses

As Se is an essential component of GPx, which plays important function in protecting cell membranes against oxidative damage by catalysing crucial reactions to convert fatty acid hydroperoxides and hydrogen peroxides into water using reduced glutathione (Lin & Shiau, 2005; Rotruck *et al.*, 1973; Watanabe *et al.*, 1997). Previous studies stated a positive relationship between dietary Se and GPx activity in fish (Le & Fotedar, 2014b; Lin & Shiau, 2005; Liu *et al.*, 2010). The increases in hepatic GPx were observed in grouper (Lin & Shiau, 2005), largemouth bass (Zhu *et al.*, 2012), gibel carp (Han *et al.*, 2011), African catfish (Abdel-Tawwab *et al.*, 2007) and yellowtail kingfish (Le & Fotedar, 2014b) fed dietary Se levels of up to 4.00, 2.06, 5.13, 5.54 and 20.87 mg/kg, respectively. However, the liver GPx gained a peak in cobia fed dietary Se of only 0.85 mg/kg (Liu *et al.*, 2010). The organic Se is known to have higher bioavailability in rising liver GPx than inorganic Se, as in common carp *Cyprinus carpio* (Jovanovic *et al.*, 1997) and channel catfish (Wang & Lovell, 1997). However, in hybrid striped bass (Cotter *et al.*, 2008) and Atlantic salmon (Bell & Cowey, 1989) indicated higher hepatic GPx and plasma GPx fed dietary selenite than fed organic Se such as Se-yeast and SeMet. This can be attributable to the complexity of chemical speciation of Se, where some Se forms may be incapable to associate with glutathione and to propagate the oxidative damage (Spallholz & Hoffman, 2002).

The positive effects of Se on immune functions in yellowtail kingfish, where the fish fed dietary organic Se as Se-yeast or SeMet showed increased bactericidal activity than the fish fed diet with no Se supplementation (Le & Fotedar, 2014a). As bactericidal activity serves as natural defense protecting the host against an invasion of microorganisms (Ueda *et al.*, 1999). Therefore, an increased organic Se can reflect the enhancement in fish immune system as observed in channel catfish (Wang & Lovell, 1997).

The hematological changes are also linked to the dietary Se level. The hematocrit (Ht) levels of yellowtail kingfish fed more than 15.43 mg/kg Se were significantly lower than the fish fed lower dietary Se levels (Le & Fotedar, 2013). This is in contradiction in African catfish, where Ht, red blood cells (RBC) and hemoglobin (Hb) levels are significantly increased as increasing dietary Se levels (Abdel-Tawwab *et al.*, 2007). Meanwhile, the RBC and Hb concentrations in black seabream showed no significant differences after being fed to 1.29 mg/kg Se, but slightly reduced in fish fed diet containing 12.3 mg/kg Se (Lee *et al.*, 2008). In animals, the RBC and Hb play important

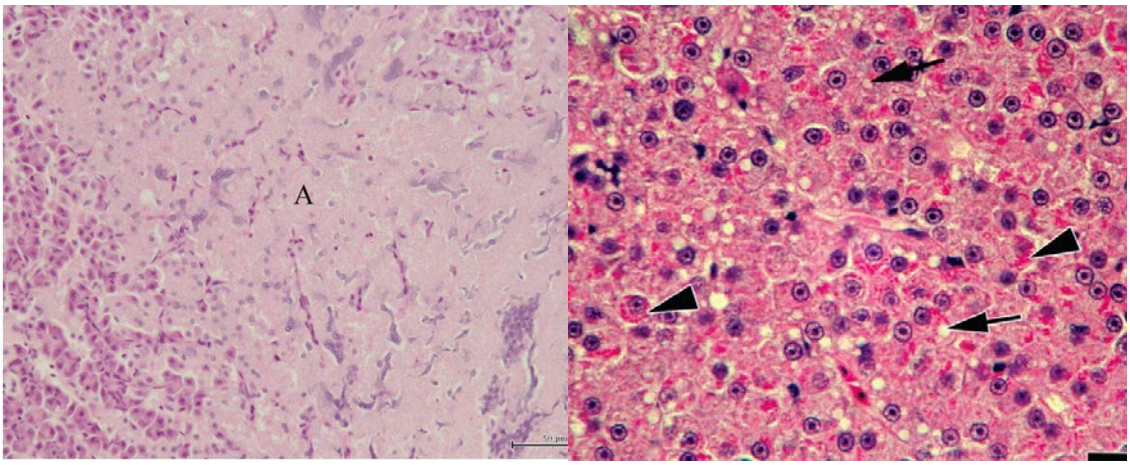
roles in transport oxygen and carbon dioxide in the blood and haemoglobin synthesis (Olugbemi *et al.*, 2010). The increased RBC and Hb can be used as indicators indicating the enhancement of fish health stimulated by dietary Se supplementation (Abdel-Tawwab *et al.*, 2007; Kader *et al.*, 2010).

Abdel-Tawwab *et al.* (2007) revealed the increases in total lipid, total protein, albumin and globulin levels, aspartate aminotransferase and alanine aminotransferase in blood of African catfish as increasing dietary Se levels. The performances of non-specific immune parameters such as albumin and globulin have been used as indicators to evaluate the effects of nutrients in fish immunity. The increase in albumin and globulin is attributed to the increases of these compounds in liver (Abdel-Tawwab *et al.*, 2007).

The interrelationship between dietary Se and histopathological alterations has been evidenced in fish, mainly due to the excessive Se concentrations in diets. However, the effects are variable, depending on different tissues, exposed Se concentrations and the species. Juvenile sacramento splittail *Pogonichthys macrolepidotus* exposed to 6.6 mg/kg Se diet for 9 months resulted in severe glycogen depletion and moderate fatty vacuolar degeneration in the liver tissues, whereas moderate eosinophilic protein droplets, mild fatty vacuolation and glycogen depletion were observed in liver tissues of fish fed 26.04 mg/kg Se diet for 5 months (Teh *et al.*, 2004). Yellowtail kingfish fed dietary of 20.87 mg/kg Se in the fishmeal-based diet caused atrophic hepatocytes (Le & Fotedar, 2014b), meanwhile, white sturgeon showed histopathological alternations such as glycogen depletion, hepatocellular vacuolar degeneration and necrosis in livers, and hepatocellular and bile ductural hyperplasia when exposed to diets containing more than 20.5 mg/kg Se (Tashjian *et al.*, 2006). Green sunfish *Lepomis cyanellus* fed dietary Se levels of 7.0 and 21.4 mg/kg dry weight, respectively, showed lymphocyte infiltration and an increase in lipid droplets relative to the fish containing 1.3 mg/kg Se in the liver (Sorensen *et al.*, 1984). No histopathological alterations were recorded in the liver tissues of olive flounder and black seabream exposed up to 18.6 and 12.3 mg/kg Se diet, respectively (Lee *et al.*, 2008; Lee *et al.*, 2010).

The cell necrosis of hepatocytes can be explained by the gradual deterioration in synthesis of new structural and metabolic component of the cell to restore the damages caused by toxic effects of Se, resulting in cell death (Teh *et al.*, 2004). Besides, glycogen depletion induced by increasing glycogenolysis may also cause single cell necrosis and macrophage aggregates in the liver. The lipid vacuolar degenerations in livers may be

results of the changing in protein turnover and lipid metabolism caused by Se toxicity, consequently, resulting in incapacitation of liver in metabolism and excretion of biochemicals (Teh *et al.*, 2004).



Hepatocyte atrophy in livers of yellow tail kingfish fed 20.87 mg/kg Se diet (Le & Fotedar, 2014b)

Moderate eosinophilic protein droplets (arrowheads) and mild fatty vacuolation (arrows) and glycogen depletion in liver of juvenile splittail fed 26.04 mg/kg Se diet (Teh *et al.*, 2004)

Figure 2.1.2 Histopathological lesions in liver tissues of fish fed high dietary Se levels

The histological changes including polycystic dilation, degeneration of tubular cells, and renal cell necrosis have also been reported in kidney tissues of fish fed high dietary levels of Se (Lee *et al.*, 2008; Lee *et al.*, 2010; Tashjian *et al.*, 2006). Juvenile olive flounder fed diets containing more than 7.38 mg/kg Se in the SeMet form for 10 weeks showed severe changes in the kidney tissue (Lee *et al.*, 2010), whereas toxic levels of dietary Se were 12.3 mg/kg in black seabream after 15 weeks exposure (Lee *et al.*, 2008) and 20.5 mg/kg or above this level in white sturgeon after 8 weeks (Tashjian *et al.*, 2006). Yellowtail kingfish fed fishmeal-based diet containing 20.87 mg/kg Se showed significant increases in macrophage aggregate in the spleen tissues (Le & Fotedar, 2014b), however, this signs was also reported in fish fed dietary Se of only 3.35 mg/kg after challenges against *Vibrio anguillarum* (Le & Fotedar, 2013). Due to the important roles of macrophage aggregate in injured cell storing (Wolke, 1992), the increased macrophage aggregate in the spleen can be used as bio-indicator to estimate the toxicity of dietary Se levels in fish.

The intestinal structures appear less susceptibility to dietary Se than other digestive organs. Le and Fotedar (2014b) reported that yellowtail kingfish fed up to 20.87 mg/kg Se diet did not show any histological alterations in the intestine after 10 weeks exposure, similar in barramundi fed plant-based diet with Se supplementation (Ilham *et al.*, 2016a).

The effects of dietary Se deficiency in histological performances in fish have been reported in a few fish species. Yellowtail kingfish fed dietary Se levels of 2.31 and 3.35 mg/kg in the fishmeal and/or commercial diets, respectively, resulted in necrotic fibres in the muscle tissues (Le & Fotedar, 2013, 2014b). Common carp fed deficient Se diets showed histopathological lesions in the tissues, resulting in muscle atrophy, degeneration and necrosis in the liver, pancreatic cells and renal tubular epithelial cells (Wang *et al.*, 2013). In contrast, muscles of black seabream and white sturgeon showed normal histopathological structures when exposed to 0.21 and 0.40 mg/kg Se diet, respectively (Lee *et al.*, 2008; Tashjian *et al.*, 2006).

2.1.7 Selection of selenium form

Selenite and selenate are inorganic forms of Se, whereas organic Se includes SeMet and SeCys (Watanabe *et al.*, 1997). Past investigations have indicated higher bioavailability of organic Se in fish than inorganic Se sources (Bell & Cowey, 1989; Le & Fotedar, 2014a; Lin, 2014; Wang & Lovell, 1997; Wang *et al.*, 2007; Zhou *et al.*, 2009), which is associated with the differences in metabolic pathway between two Se forms (Luten *et al.*, 2008). In all species, the SeMet is metabolised following the methionine pathways, where it is readily assimilated into proteins and then accumulated in liver and muscle tissues (Terry & Diamond, 2012; Yeh *et al.*, 1997), meanwhile selenite is converted to selenite before binding with albumin or hemoglobin and transported to liver for further processing (Haratake *et al.*, 2008).

Compared to inorganic Se, organic Se supplementation maintains the elevated platelet GSHPx activity for longer duration (Levander *et al.*, 1983). The higher absorption and digestibility of organic Se than the inorganic forms in yellowtail king fish (Le & Fotedar, 2014a) and channel catfish (Paripatananont & Lovell, 1997) is further example of the higher bioavailability of Se in its organic forms. Further, the SeMet and Se-yeast are beneficial Se sources in aqua-feeds due to their higher digestibility and accumulation than the SeCys, as evidenced in yellowtail kingfish (Le & Fotedar, 2014a). However, purified SeMet is not stable and is quickly oxidised, whereas SeMet in yeast shows higher stability (Block *et al.*, 2004). The SeMet in Se-yeast also accounts for 81 – 82%

of total Se with less toxicity than pure SeMet (Schrauzer, 2006). Se in Se-yeast is easily assimilated with glucomano-protein compounds in the yeast cell wall or O- and N-glucosylated proteins in the intracellular space. During feed production, the carbohydrate complexes presented in the ingredients increase the stability of these selenoproteins when feed ingredients are extruded under high temperature and pressures (Sweetman *et al.*, 2010). Besides, the beneficial effects of Se-yeast in protecting the host against disease challenges and histopathological lesions have also been reported when it is used to replace the inorganic Se form (Sweetman *et al.*, 2010). Therefore, Se-yeast was selected as a Se source to evaluate the effects of Se supplementation on snapper and cobia in this study.

2.2 LUPIN MEAL PRODUCTION AND USE IN AQUACULTURE

2.2.1 Lupin production

Australia accounts for more than two-third of the total lupin production in the world (Kaushik & Hemre, 2008). Narrow-leafed lupin, white lupin and yellow lupin are three major commercial lupin species, with the majority of production being the narrow-leafed lupin, comprising 77 % of global production. The total lupin production in Australia was approximately 1 million tons in 2012 which was mainly produced in Western Australia, accounting for 782.000 tons (Statistics, 2013). However, the total area recently produced lupin had reduced to 689,000 hectares in whole Australia, especially in New South Wales (Statistics, 2013).

2.2.2 Nutritional values of lupin

Protein and amino acids

Protein content in lupin seeds range from 31-42%, depending on the different lupin species, growing conditions and soil type (Petterson *et al.*, 1997). The highest protein level was found in yellow lupin, ranging from 40 to 45 % of the whole seed, whereas narrow-leafed and white lupins contain relatively lower protein concentrations of 32 – 44 and 30-41 %, respectively (Glencross, 2001). The dehulled process significantly improves the protein content in both narrow-leafed and yellow lupins, but not for white lupin due to the thinner seed coat of this species. The protein concentrations of narrow-leafed kernel meal and yellow kernel meal are approximately 53 % and 41 %, respectively (Glencross, 2001).

Table 2.2.1 Chemical composition (% or MJ/kg) of lupin kernel meal species, soybean meal and fishmeal

Nutrient	Narrow-leafed LKM	White LKM	Yellow LKM	Soybean meal	Fishmeal
Crude protein	39.0	40.0	52.5	47.81	64.0
Crude lipid	6.82	11.4	7.2	0.70	10.70
Gross energy	20.93	20.4	20.0	20.09	20.09
Dry matter	87.61	90.5	91.7	88.91	91.43
Ash	2.41	3.3	4.3	6.77	21.90
Total calcium	0.22	0.20	0.22	0.39	n/r
Total phosphorous	0.30	0.36	0.51	0.89	n/r
Selenium (mg/kg)	0.35	0.02-0.36	n/r	n/r	4.47

Data derived from Glencross (2001), Petterson *et al.* (1997). n/r not reported.

Table 2.2.2 Essential amino acid composition (g/16 g N) in lupin species and other feed ingredients

Amino acids	Narrow-leafed lupin	Yellow lupin	White lupin	Soybean meal	Rapeseed meal	Fishmeal
Arginine	11.62	12.20	11.30	7.4	6.0	5.8
Cysteine	1.36	1.34	2.28	1.5	2.4	0.8
Histidine	2.57	1.86	3.30	2.6	2.6	2.4
Isoleucine	3.91	3.80	2.70	4.6	4.0	4.3
Leucine	6.61	6.90	7.89	7.4	6.7	7.2
Lysine	4.66	4.75	5.35	6.1	5.3	7.5
Methionine	0.72	0.66	0.70	1.4	2.0	2.8
Phenylalanine	3.65	3.85	4.04	5.0	3.9	3.9
Threonine	3.54	3.29	3.51	3.9	4.3	4.2
Tryptophan	1.00	0.97	n/r	1.3	1.2	1.0
Tyrosin	3.66	4.26	3.10	3.3	2.9	3.0

[a] Data derived from Petterson *et al.* (1997) and Kaushik and Hemre (2008), n/r: not reported.

Lupin kernel meal (LKM) contains favourably amino acids concentrations than the soybean, resulting in high concentrations of arginine, leucine and phenylalanine. Lysine, methionine, cysteine and taurine are limiting amino acids in lupin meal (Glencross, 2001). The innovation in breeding to increase the methionine content has been achieved for narrow-leafed lupin (Molvig *et al.*, 1997). The chemical composition and amino acid profiles of lupin are presented in Table 2.2.1 and Table 2.2.2.

Lipids and fatty acids

The lipid content in lupin is variable, depending on the lupin species and cultivars. The lowest crude lipid is found in *L. atlaticus* at 14 g/kg dry matter, whereas *L. mutabilis*

contains 230 g/kg DM. The lipid levels in three major lupin species including narrow-leafed, yellow and white lupin are 62-83 and 82-145 g/kg DM, respectively (Petterson, 2000). Triacylglycerides are predominant in crude fat, comprising 71.1 %, whereas phospholipids, free sterols, glycolipids, sterol and wax esters, free fatty acids and hydrocarbons account for 14.9, 5.2, 3.5, 0.5, 0.4 and 0.4 %, respectively (Petterson, 2000).

White lupin contains relatively higher monounsaturates (59.2 %) than those in narrow-leafed (38.5 %) and yellow lupin (23.6 %), however, total polyunsaturates (PUFA) is only 26.7 %, considerably lower than that of narrow-leafed lupin (42.4 %) and yellow lupin (54.8 %). White lupin contains highest level of n-3 (omega-3) (9.5 %), followed by yellow lupin and narrow-leafed lupin (7.5 and 5.3 %, respectively). In contrast, the highest levels of n-6 (omega-6) is found in yellow lupin (47.3 %), followed by narrow-leafed lupin (37.1 %) and white lupin (17.2 %) (Glencross, 2001).

The total carotenoids in lupin varies considerably in different lupin species, resulting in lowest level in white whole seed lupin (9 mg/kg) and highest in narrow-leafed lupin (35 mg/kg). Hydroxycarotenoids is the major carotenoids in lupin, accounting for 66 % of total carotenoids in narrow-leafed lupin, whereas β-carotene comprises about 30 % of all carotenoids (Glencross, 2001). However, astaxanthin, an important carotenoid required in fish, is deficient in lupin (Glencross, 2001).

Vitamin and mineral contents

The concentration of minerals in lupin vary slightly among different lupin species and the soil conditions where lupin was produced (Petterson, 2000). The major minerals in lupin seeds comprise calcium, magnesium, phosphorus, potassium, sodium and sulphur, whereas copper, iron, manganese, molybdenum, zinc, cobalt and selenium account for minor amount in lupin ingredients (Table 2.2.3).

Petterson (2000) reported the levels of vitamins in the whole seed meal of narrow-leafed lupin as: thiamin (5.9 mg/kg), riboflavin (3.1 mg/kg), biotin (0.04 mg/kg), folate (0.4 mg/kg), choline (3.4 mg/kg), niacin (40 mg/kg), pantothenate (1.8 mg/kg), α-tocopherol (2.4 mg/kg) and β-carotene (3.9 mg/kg).

Table 2.2.3 Mineral concentrations in lupin species

Minerals	Narrow-leafed lupin	White lupin	Yellow lupin

Major minerals (g/kg)			
Calcium	1.5 – 2.9	1.2 – 2.5	1.8 – 2.6
Magnesium	1.1 – 2.0	0.9 – 1.6	2.2 – 3.2
Phosphorus	2.1 – 4.3	2.5 – 9.0	3.4 – 6.0
Potassium	6.6 – 9.1	2.8 – 11.1	8.8 – 10.0
Sodium	0.3 – 1.1	0.1 – 0.3	< 0.1
Sulphur	1.5 – 2.9	2.1 – 2.7	4.0 – 4.9
Minor minerals (mg/kg)			
Copper	3 - 7	3 - 8	6 - 12
Iron	31 - 150	21 - 44	52 - 70
Manganese	7 - 76	23 - 3772	25 - 50
Molybdenum	1 - 3	1 - 3	n/r
Zinc	25 - 45	22 - 38	39 - 82
Cobalt (µg/kg)	10 - 260	10 - 430	n/r
Selenium (µg/kg)	18 - 240	20 - 360	n/r

Data derived from Petterson (2000), n/r not reported.

Anti-nutrients in lupin and their nutritional effects in fish

Alkaloids are predominant anti-nutrient in lupin products, however the selective breeding recently have reduced the alkaloids levels in lupin to insignificantly levels of less than 0.1 g/kg dry matter which can be readily consumed by animals (Gatlin *et al.*, 2007; Glencross, 2001). The alkaloids in narrow-leafed lupin comprise lupinine (42-59 %), 13-hydroxylupanine (24-45 %) and angustifoline (7-15 %). A very little information is available on the nutritional effects of alkaloids in fish. Diets containing more than 1000 mg/kg alkaloids can result the palatability problems (Glencross, 2001), whereas the increasing dietary lupinine or spartein levels up to 5000 mg/kg had no effects on tissue histopathology of rainbow trout (Serrano *et al.*, 2012; Serrano *et al.*, 2011).

The oligosaccharides including stachyose, sucrose, verbascose and raffinose vary in different lupin strains, growing seasons and analyzed methods, and range from 41 g/kg in narrow-leafed lupin to 89 g/kg in white lupin (Petterson, 2000). The presence of oligosaccharides in diets can cause deleterious effects on digestive process due to their binding to bile acids or interference with the digestive enzymes or substrate transport in the intestine (Glencross *et al.*, 2003a; Storebakken *et al.*, 1998). The reduced digestibility associated to oligosaccharides in narrow-leafed lupin has been reported in rainbow trout (Glencross *et al.*, 2003a), whereas the reduction in growth and digestibility

in Atlantic salmon was attributable to the presence of oligosaccharides in soybean-based diets (Refstie *et al.*, 1998).

Lupin meal contains insignificant concentration of phytate (0.5 %) than in soybean (1-1.5 %) and rapeseed meal (5-7.5 %). The chelation of phytate presented in diet and minerals such as calcium and zinc can reduce the uptake and metabolism of these nutrients (Antony Jesu Prabhu *et al.*, 2016; Kumar *et al.*, 2012). However, the anti-nutritional effects of phytate may vary, depending on its content in the diet and the existence or absenteeism of distinct stomach (Usmani & Jafri, 2002). The rohu *Labeo rohita* and mrigal *Cirrhinus mrigala* showed reduced SGR when fed diets containing more than 10 g/kg of phytate (Usmani & Jafri, 2002). Atlantic salmon fed dietary phytate of 18 g/kg resulted in the reductions of growth and mineral utilization (Storebakken *et al.*, 1998) , whereas this level were 22 and 13 g/kg in channel catfish (Satoh *et al.*, 1989) and striped bass *Morone saxatilis* (Papatryphon *et al.*, 1999), respectively. The reduced protein and amino acid digestibility and histopathological lesions in the digestive tissues were also reported in fish and animals fed diets containing high levels of phytate (Antony Jesu Prabhu *et al.*, 2016).

The saponin content in lupin considerably varies among lupin species. The narrow-leafed lupin seed contains 0.57 g/kg, while this concentration is only 0.055 g/kg in the yellow lupin (Glencross, 2001). The presence of saponins in diets can increase the permeability of the small intestine mucosal cells (Glencross, 2001) and reduce the transportation of nutrients (Francis *et al.*, 2001). The reduced growth performances of rainbow trout and tilapia had been linked to the high inclusion levels of saponins in their diets (Francis *et al.*, 2001). Interestingly, the integration between saponins and other anti-nutritional factors could inactivate the negative effects of these components. The toxicities of saponins and tannins could be inactivated by the linkage of saponin- tannins, as reported in animal (Freeland *et al.*, 1985). Besides, almost saponins can be extracted from the plant-derived ingredients due to their high solubility in water, making these feedstuffs have little negative effects in fish (Francis *et al.*, 2001).

The binding between tannins and enzymes and/or nutrients in diets such as protein or minerals can interfere the nutrient digestion, reduce the vitamin absorption (Francis *et al.*, 2001), as described in common carp (Becker & Makkar, 1999). Similar to saponins, the interaction between tannins and cyanogenic glycosides in the diets can reduce the detrimental effects of the latter (Goldstein & Spencer, 1985), while the impacts of

tannins on amylase can be eliminated due to the interaction between tannins and lectins (Fish & Thompson, 1991). However, the concentration of tannins in lupin (0.1 – 0.3 g/kg) is very low in comparison to those in rapeseed meal (12 g/kg), which are not likely to have anti-nutritional effects in fish (Glencross, 2001; Petterson, 2000).

Table 2.2.4 Anti-nutrient concentrations in narrow-leafed lupin and other plant-derived ingredients

Anti-nutrient (g/kg)	Yellow lupin whole seed	White lupin whole seed	Narrow-leafed whole seed	Narrow-leafed LKM	Rapeseed meal	Defatted soybean meal
Trypsin inhibitor	0.16	0.08	0.12	n/r	n/r	3.11
Phytate	9.3	5.7	5.0	5.4	31	15.9
Saponins	n/r	n/r	0.57	n/r	n/r	n/r
Alkaloids	< 0.50	< 0.10	< 0.20	< 0.12	n/r	n/r
Oligosaccharides	89	66	41	77	n/r	52
Tannins	0.30	0.20	0.10	1.7	12	n/r

Data derived from Glencross (2001), Luo *et al.* (2012) and Vo *et al.* (2015).

Lupin meal contains insignificant concentrations of protease inhibitors. The levels of trypsin and chymotrypsin inhibitors are 0.3 and 0.6 mg/kg, respectively in narrow-leafed lupin seed (Petterson *et al.*, 1997), considerably lower than those in other plant-derived ingredients such as soybean containing 60 g/kg in unprocessed seed and 3.4 g/kg in soybean meal (Glencross, 2001). The concentrations of anti-nutritional factors in lupin species are presented in Table 2.2.4.

2.2.3 Use of lupin in aqua-feeds

Meals for narrow-leafed lupin, white lupin and yellow lupin can be used as alternative protein sources to replace protein from fishmeal in fish diets due to their high protein contents, reasonable prices and easy availabilities (Pereira & Oliva-Teles, 2004; Salini & Adams, 2014). A series of studies have been conducted to evaluate the nutritional effects of lupin meal as alternative protein ingredient for fishmeal replacement in aqua-feeds for salmonids (Borquez *et al.*, 2011; Glencross *et al.*, 2004b; Refstie *et al.*, 2006), gilthead seabream (Pereira & Oliva-Teles, 2004; Robaina *et al.*, 1995), barramundi (Ilham *et al.*, 2016a; Tabrett *et al.*, 2012), Australian snapper (Glencross & Hawkins, 2004) with varied results, depending on lupin species, cultivars and lupin-protein products. Most authors stated that lupin meals can partially replace of the fishmeal protein in aqua-feeds, however, high inclusion levels of lupin results in reduction in growth and feed efficiencies, adverse physiological function and histopathological lesions in the host fish

tissues. An overview of lupin as alternative protein source to replace fishmeal in various fish is described in Table 2.2.5.

Effects of dietary lupin on growth and feed utilisation

Farhangi and Carter (2001) and Glencross *et al.* (2004b) showed that up to 400 g/kg lupin of narrow-leafed and yellow lupins can be successfully used in rainbow trout, without impairing growth and nutrient utilisation, however, the reduced growth and energy efficiency ratio were observed in fish fed to 500 g/kg lupin. Meanwhile, up to 500 g/kg of whole grain white lupin could be included in the diet of this species without reducing growth and feed efficiency (Borquez *et al.*, 2011). The increasing dietary narrow-leafed lupin kernel meal from 0 to 510 g/kg had no negative effects on growth, feed intake and FCR in barramundi (Ilham *et al.*, 2016a). The barramundi fed 300 g/kg yellow or narrow-leafed lupin kernel meal resulted in significantly higher final weight and feed intake than those fed fishmeal-based diet (Glencross *et al.*, 2011). Gilthead seabream also showed less susceptibility to dietary lupin, where approximately 400 g/kg lupin seed meals could be incorporated without depressing growth and feed utilisation (Pereira & Oliva-Teles, 2004; Robaina *et al.*, 1995).

Nutrient digestibility

As lysine and methionine are limiting essential amino acids in lupin meal, high inclusion levels of lupin in diets can result in imbalanced amino acid profiles (Gatlin *et al.*, 2007), consequently, impairing the growth and feed utilisation in fish (Borquez *et al.*, 2011). Besides, the presence of anti-nutritional factors in lupin might interfere with other nutrients, resulting in low digestibility of these components. Glencross and Hawkins (2004) demonstrated a higher digestibility of protein (> 88 %) and phosphorus (> 90 %) in both Australian snapper and rainbow trout fed different lupin species, whereas the organic matter and energy were poorly digested, with less than 60 % and 70 %, respectively. This finding was similar to barramundi (Ilham *et al.*, 2016a; Tabrett *et al.*, 2012).

However, the nutrient digestibility of lupin meal shows a degree of variability, depending on the lupin species, cultivars and lupin products (Glencross, 2001; Tabrett *et al.*, 2012). The digestibility of organic matter and energy in barramundi fed white lupin kernel meal were significantly higher than in fish fed narrow-leafed lupin kernel meal (Tabrett *et al.*, 2012). Rainbow trout fed lupin protein isolates resulted in higher nutrient

digestibility than fed lupin kernel meal or lupin protein concentrates (Glencross *et al.*, 2010), whereas dietary lupin protein concentrates resulted in higher digestibility of protein, organic matter and energy values in Atlantic salmon than fish fed lupin kernel meal (Refstie *et al.*, 2006).

Borquez *et al.* (2011) showed that rainbow trout fed dietary whole grain white lupin did not cause any effects on proximate composition in the whole-body and muscle fatty acid concentrations, similar to those reported in rainbow trout fed to yellow lupin kernel meal (Glencross *et al.*, 2004b) or narrow-leafed lupin kernel meal, except a slight fluctuation in crude protein level (Farhangi & Carter, 2001). This was also consistent with the results reported in gilthead seabream (Pereira & Oliva-Teles, 2004) and Atlantic salmon (Carter & Hauler, 2000) after being fed to narrow-leafed lupin seed meal and/or narrow-leafed protein concentrate, respectively. However, the reduction in crude lipid caused by high dietary narrow-leafed lupin seed was reported in gilthead seabream (Robaina *et al.*, 1995), which could be attributed to a low energy intake caused by a low feed intake, energy digestibility in fish fed high inclusion level of plant-derived ingredients (Luo *et al.*, 2012).

High inclusion levels of plant-derived feedstuffs in aqua-feeds may result in imbalanced amino acid profiles in the diets due to the low concentrations of essential amino acids as lysine, methionine in plant meal (Gatlin *et al.*, 2007), thus, impairing the amino acid concentrations in fish, as reported in cobia (Chou *et al.*, 2004) and gilthead seabream (Gómez-Requeni *et al.*, 2004). However, there is no available information on the effects of dietary lupin on amino acid profiles in tissues of fish.

Effects of dietary lupin on the physiology of the host species

Barramundi fed dietary levels of 185 and 510 g/kg lupin kernel meal did not show any significant changes in haematological parameters and GPx activity in RBCs after 60 days exposure (Ilham *et al.*, 2016a). Dietary lupin also did not affect the total cholesterol and triacylglycerol in plasma of black seabream (Zhang *et al.*, 2012). As RBC and Hb play important roles in fish health (Kader *et al.*, 2010), the levels of RBC and Hb are useful bio-markers to evaluate health status in fish fed lupin-based diets.

Dietary lupin also resulted in the reduction of protease and trypsin activity in gilthead seabream (Glencross *et al.*, 2004b). However, these effects are species-specific and dependent on different lupin ingredients. Refstie *et al.* (2006) investigated the

physiological responses of Atlantic salmon fed to different lupin products, wherein, fish fed yellow and narrow-leafed protein concentrates resulted in significantly higher trypsin activity and bile acid concentrations in the distal intestine than fed lupin kernel meals. Dietary lupin had no effects on hepatosomatic index (HSI) and viscerasomatic index (VSI) in gilthead seabream (Glencross *et al.*, 2004b; Pereira & Oliva-Teles, 2004) and black seabream (Zhang *et al.*, 2012). This was opposite to the reduced HSI in rainbow trout fed high levels of whole grain lupin (Borquez *et al.*, 2011).

The histopathological lesions including the increase in lipid droplet in the livers, changes in the structural muscles caused by dietary lupin meal levels have been observed in fish (Gu *et al.*, 2014; Ilham *et al.*, 2016a). The falsification, disconnection, and longitudinal rupture of muscle fibres were observed in barramundi fed 185 and 510 g/kg narrow-leafed lupin kernel meal, whereas fish fed 510 g/kg lupin also showed increasing hepatic lipid droplets (Ilham *et al.*, 2016a). The increased lipid vacuolization was also found in hepatocytes of rainbow trout fed dietary inclusion of 400 g/kg whole grain white lupin (Borquez *et al.*, 2011), similar to reported in gilthead seabream fed 208 g/kg narrow-leafed lupin seed (Glencross *et al.*, 2004b). Atlantic salmon fed diets containing 200 g/kg lupin meal and 190 g/kg wheat gluten showed increased lipid droplet deposition in enterocytes and severe hepatic steatosis compared to those fed fishmeal-based diets (Gu *et al.*, 2014), which may be linked to increasing lipolysis from adipose tissue, as mentioned in Atlantic salmon (Gu *et al.*, 2014) and gilthead seabream (Bouraoui *et al.*, 2011).

The increment of lipid accumulated in liver tissues have been linked to deficiency of Se in the diets (Burk *et al.*, 1995). Lupin meal contains insignificant concentration of Se (Petterson, 2000), which can lead to Se deficiency when high fishmeal protein is replaced with lupin protein ingredient, resulting in histological changes in liver tissues, as reported in barramundi (Ilham *et al.*, 2016a). The barramundi fed lupin-based with organic Se supplementation did not show any histopathological lesions in the livers (Ilham *et al.*, 2016a), however, the specific mechanism of the interaction between Se and plant-derived ingredients in histological responses in fish is still unknown.

The increment of lipid accumulations in intestines induced by dietary lupin meal have been demonstrated in Atlantic salmon (Gu *et al.*, 2014) and rainbow trout (Borquez *et al.*, 2011) fed 200 and 500 g/kg, respectively of lupin meals. This is inconsistent with a report in Atlantic salmon, where fish fed 300 g/kg lupin kernel meal showed normal

intestinal morphology, resulting in well-differentiated enterocytes with many absorptive vacuoles. Similar results were also observed in digestive tracts of barramundi (Ilham *et al.*, 2016a) fed different inclusion levels of lupin kernel meal.

Table 2.2.5 Overview fishmeal replacement by lupin meals in diet for fish

Species	Lupin protein sources	Inclusion rate (g/kg)	Optimum inclusion level (g/kg)	Protein in control diet (%)	FM in control diet (g/kg)	Negative effects of high inclusion levels	References
Rainbow trout *Oncorhynchus mykiss*	Whole grain white lupin	300 - 500	500	49.13	400	Slight changes in muscle fatty acids	Borquez *et al.* (2011)
Rainbow trout *Oncorhynchus mykiss*	Yellow lupin kernel meal	125 - 500	125	51.6	650	Reduced growth, feed efficiency and minor changes in liver tissues	Glencross *et al.* (2004b)
Rainbow trout *Oncorhynchus mykiss*	Dehulled lupin	100 - 500	400	44.15	611.9	Reduced growth and feed efficiency	Farhangi and Carter (2001)
Gilthead seabream *Sparus aurata*	Narrow-leafed lupin seed meal raw	132.6 – 297.6	300	45.56	638.8	Decreased growth	Pereira and Oliva-Teles (2004)
Gilthead seabream *Sparus aurata*	Micronized narrow-leafed lupin seed meal	253 – 380	380	45.56	638.8	No record	Pereira and Oliva-Teles (2004)
Gilthead seabream *Sparus aurata*	Lupin seed meal	115 – 346	< 200	55.78	766.1	Reduced body lipid and trypsin activity, increased lipid deposition	Robaina *et al.* (1995)
Atlantic salmon *Salmo salar*	Narrow-leafed lupin concentrate	218 - 292		39.2	601.5	Reduced growth	Carter and Hauler (2000)
Atlantic salmon *Salmo salar*	Tasmanian white lupin kernel meal	250	250	44	621	Reduction in growth and feed efficiency in fish fed Luxor cultivar	Salini and Adams (2014)
Barramundi *Lates calcarifer*	Narrow-leafed lupin kernel meal	185 and 510		49	460	Histological damages in muscle and liver tissues.	Ilham *et al.* (2016a)
Cobia *Rachycentron canadum*	Narrow-leafed lupin kernel meal	105 - 420	210	46	320	Reduced growth, feed efficiency and haematological responses	Pham *et al.* (2016)

2.3 SNAPPER AND COBIA TAXONOMY AND BIOLOGY

2.3.1 Snapper

Snapper was previously termed as *Chrysophrys auratus* and *C. unicolour* in Australia, *Chrysophrys* in New Zealand and *P. major* or *C. major* in Japan (Paulin, 1990) (Figure 2.3.1). Based on the morphology, Paulin (1990) evaluated the synonym of *Chrysophrys* is *Pagrus* and re-described *C. auratus* as *P. auratus.* Paulin (1990) also confirmed that that *P. major* was a conspecific and should be re-described as *P.auratus*. Whereas, Tabata and Taniguchi (2000) proposed the relation between *P.auratus* and *P.major* at subspecies level based on the differences in DNA divergence levels, in which *P. major* may be renamed as *P. auratus major*, while *P. auratus* can be re-described as *P. auratus auratus*, distributed in southern coastline of mainland Australia and New Zealand (Quartararo *et al.*, 1998a; Tabata & Taniguchi, 2000). The snapper in this thesis are referred to *P. auratus*, and following taxonomy is followed (Species 2000 & ITIS Catalogue of Life, 2013a).

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Perciformes
Family	Sparidae
Genus	Pagrus
Species	*Pagrus auratus* Forster, 1801

Snapper is widely distributed in warm temperate water of Indo-Pacific region at depth ranged from 0 to 200 m (Paulin, 1990). This species are also found in temperate to subtropical waters in southern hemisphere and temperate to tropical waters in northern hemisphere, but not distributed from Pacific Plate (Paulin, 1990). Juvenile snapper, in the natural environment mainly live in estuaries, and migrate to coastal and offshore waters as adults (Fielder *et al.*, 2001). In warm water, snapper generally gain maturity in three or four years at 40 cm long, whereas fish in cooler waters reach maturity after 5 or 6 years at 60 cm long.

Figure 2.3.1 Australian snapper *Pagrus auratus* (Species 2000 & ITIS Catalogue of Life, 2013a)

The spawning season of snapper may vary with location, depending on water temperature. Snapper spawn from April to October in warm-waters and from October to December in cooler waters. Brooders with 40 cm long can produce 100,000 eggs in a spawning, whereas bigger broodstocks with 70 cm can release 300,000 to 700,000 eggs in a single spawning (Department of Fisheries, 2011). Snapper are carnivorous fish, and can eat small fish, crustaceans, worms, molluscs, jellyfish, echinoderms (such as sea stars and sea urchins) and algae. The optimum temperature for growth of snapper ranges from 22 to 26 ^{0}C, and start showing deaths when temperature is higher than 32 ^{0}C (Partridge *et al.*, 2003). Snapper have slow growth rate than other species, making them less able to recover quickly from overexploitation and environmental changes (Department of Fisheries, 2011).

2.3.2 Cobia

Cobia, marine pelagic carnivore fish, is widely distributed in subtropical, tropical and temperate waters, except in the eastern Pacific (Briggs, 1960) (Figure 2.3.2). This species inhabits in estuaries, bay, coastal to offshore salt waters with optimum temperature ranged from 25 – 32 ^{0}C, although specimens might be found in lower temperature. Cobia shows less sensitive to salinity, wherein the fish can grow in wide range of salinity from 5 to 44.5 ‰ (Kaiser & Holt, 2005).

The spawning season may change, depending on the water quality in different regions. Brooders in Taiwan breed spontaneously (Liao *et al.*, 2004), whereas broodstocks in

southern United States mainly spawn from April to September with mean fecundity ranges from 377,000 to 1,980,000 eggs (Brown - Peterson *et al.*, 2001). There is a positive interrelationship between fecundity with fork length. In northeastern Australia, the mean fecundity ranges from 577,468 to approximately 7,400,000 eggs. The relative fecundity of cobia have no relation with fork length, however, the total number of eggs released states a positive relationship with fork length (van der Velde *et al.*, 2010).

The taxonomy classification of cobia is as follows (Species 2000 & ITIS Catalogue of Life, 2013b)

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Perciformes
Family	Rachycentridae
Genus	Rachycentron
Species	*Rachycentron canadum* Linnaeus, 1766

Figure 2.3.2 Cobia *Rachycentron canadum* (Species 2000 & ITIS Catalogue of Life, 2013b)

Zooplankton, especially copepods are the major food for larvae cobia. During juvenile and adult stages, cobia feed mainly on benthic/epibenthic crustaceans and small fish with 77.6 % and 20.3 % of crustaceans and fish, respectively were identified in cobia's stomach in the Gulf of Mexico (Meyer & Franks, 1996). Larger cobia tend to feed fish

than other preys, resulting in 84.4 % of fish on stomach of cobia (1,150 – 1,530 mm folk length), however, the feeding habits of cobia may change, depending on the availability of preys rather than specific food organisms (Meyer & Franks, 1996).

Cobia have relatively fast growth rate in the first two year, and gradually reduced in following years. Juveniles (30 g) can reach to 5 kg in one year in sea-cage farming (Chou *et al.*, 2001). The maximum length, weight and age of female sampled in the Gulf of Mexico were 160 cm, 62.2 kg and 11 years, respectively (Franks *et al.*, 1999). The relationship between fork length with age based on the von Bertalanffy equation for male and female are $FL_t = 1171(1\text{-exp }[-0.432(t + 1.1150)])$ and $FL_t = 1555(1\text{-exp }[-272(t + 1.254)])$ (Franks *et al.*, 1999).

2.4 SNAPPER AND COBIA AQUACULTURE AND NUTRTIONAL RESEARCH

2.4.1 Snapper aquaculture

Snapper was first made to reproduce in Australia and New Zealand in the late 1980's. Some hatcheries were established in New South Wales, South Australia and Western Australia to produce fingerling for research and small-scale farming of snapper (Battaglene, 1996). Hatchery manual for Australian snapper production has been produced by Battaglene and Talbot (1992) and Partridge *et al.* (2003). Adult snapper are collected from wild and transferred to hatchery for reproduction. If fish is collected in deep water (> 15 m), the venting needs to be applied to maintain their buoyancy. Broodstock are fed prawn, squid, whitebait, mussels and pilchards at ratio of 2:2:1:1:1 to provide sufficient DHA, EPA, vitamin, carotenoids and astaxanthin requirements (Partridge *et al.*, 2003). Brooders are injected with human chorionic gonadotropin (HCG) at 1000 UI/kg and transferred into seawater through flowing tank and kept in the dark in 24 h before egg stripping. However, hatchery-reared broodstock can naturally spawn throughout the year without application of induced hormones or ecological factors such as photoperiod and temperature. Spawned eggs are 920 to 1000 µm in diameter with single oil globule ranging from 200 to 250 µm (Partridge *et al.*, 2003). During incubation, tanks need to be darkened to improve swim-bladder inflation (Battaglene & Talbot, 1992). Enriched rotifers following with brine shrimp are used to feed snapper larvae. Feeding regimes of snapper followed the protocols installed for reproduction of red seabream in Japan (Battaglene & Talbot, 1992). The larvae start weaning after 16 days with size from 7.0 mm and completed in 30 days. The commercial feeds having 200 µm in diameter can be used at this stage (Partridge *et al.*, 2003).

Juveniles (1- 20 g) are reared in tanks at a stocking density of 11.6 kg/m^3. A water flow and pure oxygen should be installed to increase water quality. As snapper is very sensitive to lighting, the illumination needs to be kept constant at the levels of at least 100 – 250 lux. During this phase, juveniles are fed commercial salmon sinking pellets containing 50 % protein and 14 % lipid. The high-energy feeds should be used for larger juveniles (40 – 60 g). Juveniles must be graded to avoid cannibalism (Partridge *et al.*, 2003).

In Australia, small-scale tank-based culture of snapper is practices mainly in New South Wales, South Australia and Western Australia because of the lack of available protected coastal areas for sea cage culture (Patridge & Jenkins, 2003), the reliance on high cost commercial diets of other marine fish (Booth *et al.*, 2005). Wild snapper juveniles are easily adapted to rearing condition and artificial feed. Snapper cultured under captivity in tanks shows significantly higher growth rate than those in natural condition. The snapper juveniles can reach to marketable size (25 cm) after 21 months (Bell *et al.*, 1991). Australian snapper can be cultured in saline groundwater if the potassium is supplemented to meet 50 % or 100 % potassium level in the coastal water (Fielder *et al.*, 2001).

Snapper cultured under poor conditions may be infested with bacteria such as *Vibrio harveyii, V. damsel, V. tubiashi, Photobacterium damsel, Flexibacter sp and Pseudomonas sp* in which fish infested *V. damsel* caused 100 % mortality in infected tank. Other diseases caused by protozoan, metazoan and parasitic copepods are also reported in snapper farming (Partridge *et al.*, 2003).

2.4.2 Cobia aquaculture

The small-scale cobia culture began in Taiwan from 1970s, relying on juveniles collected form wild. The successful seed production in 1997 has increased the cobia farming in both area and production (Liao *et al.*, 2004). The global cobia production has rapidly increased from 9 tons in 1997 to approximately 30,000 tons in 2007 (Nhu *et al.*, 2011) and 40,000 tons in 2010 (FAO, 2012). The cobia farming has been introduced to more than 23 countries and territories, mainly located in Asian-Pacific region, where China, Taiwan and Vietnam are the largest cobia producers in the world (Nhu *et al.*, 2011).

The protocol for cobia seed reproduction has been demonstrated by Liao *et al.* (2004) and Nhu *et al.* (2011). Broodstock are fed trash fish at a feeding rate of 3 – 5 %/day, and supplemented with squid oil, vitamin and minerals three months before spawning. Brooders fed commercial diets should be supplemented with n-3 HUFA to improve the egg quality and fertilisation (Nhu *et al.*, 2011). Cobia can spawn spontaneously year around, depending on water temperature. In North Vietnam, cobia spawn naturally from April to July, whereas it spawns from January to November in South Vietnam (Nhu *et al.*, 2011). Hormone, LRH-a can be used to induce cobia breeding at doses of 20 μg/kg for female and 10 μg/kg for male. Cobia larvae can be reared in intensive system using recirculating water system and live feed products or in pond with natural zooplankton, as detailed description by Nhu *et al.* (2011). To improve the growth and survival, rotifers and *Artemia* need to be enriched to provide adequate n-3 HUFA requirement for cobia larvae (Nhu *et al.*, 2011).

The cobia is usually cultured in sea-cages. In Vietnam, the small-scale wooden cages (4 x 4 x 4m) are installed in closed bays while trash fish are used as the major feed (Nhu *et al.*, 2011). In Taiwan, cobia fingerlings are stocked in both rectangular cages (3 x 3 x 3m) in small-scale circular cages (7 x 5m) before transferred into bigger circular cages (12.7 x 8m or 16 x 9m) (Liao *et al.*, 2004). The commercial diets containing 42 – 45 % crude protein and 15 – 16 % lipid are generally used in grow-out cobia farming in Taiwan. After 12 -14 months, cobia can reach to 7 kg with FCR of 1.5 (Liao *et al.*, 2004). In Vietnam, cobia fed extruded pellet diet can reach to 6.84 kg after one year, is twice higher than those fed trash fish (3.35 kg). The FCR of cobia fed commercial feeds varies from 1.2 – 2.0, depending on fish size, but lower than those fed trash fish (2.4) in dry weight (Nhu *et al.*, 2011).

During larval rearing, protozoan infestations from *Vorticella sp*, *Epistylis sp*, *Benedenia Pseudorhabdosynochus epinepheli*, and *Trichodina* have been reported, whereas *Amyloodinium ocellatum* and *Myxosporidean* infestation may cause massive mortality in cobia juveniles (Liao *et al.*, 2004; Nhu *et al.*, 2011). The bacterial infections can cause high mortality in cage culture of cobia. Besides, the quantity and quality of cobia juveniles, availability of commercial feeds and market as well as weather conditions are major challenges for expansion of cobia farming (Liao *et al.*, 2004; Nhu *et al.*, 2011).

2.4.3 Nutritional research on snapper

A very little information on the nutritional requirement for Australian snapper is available. Thus, the commercial barramundi and Atlantic salmon feeds (51.9 % crude protein and 13.5 % lipid) are generally used to feed snapper both in commercial and laboratory environment (Booth *et al.*, 2012). Booth *et al.* (2007) determined digestible protein (DP) requirement in Australian snapper fed various levels of digestible energy (DE). The optimum digestible protein to energy ratio in fish fed high, mild and low energy diets are 460:20, 420:18 and 350:15, respectively, whereas the optimum DP:DE ratio for maximum growth and protein deposition in this species is 23 g (Booth *et al.*, 2007). Glencross *et al.* (2004d) also indicated that the Australian snapper fed diet containing 52.6 % crude protein showed significantly higher growth rate than those fed diet containing 36 % protein. There is no published report on amino acid requirement of Australian snapper. Whereas, the dietary lysine and taurine requirement for subspecies, red sea bream *Pagrus major* have been quantified to be 3.6 and 0.52 % of the diet, respectively (Forster & Ogata, 1998; Matsunari *et al.*, 2008) which can be applicable in formulated diet for Australian snapper. Moderate levels of gelatin starch can be digested by Australian snapper, however, the starch levels of 250 and 350 g/kg are threshold levels for small (110 g/fish) and large (375 g/fish) snapper (Booth *et al.*, 2006).

In spite of the lack of standard reference diet, a few studies on the digestibility and growth performance of snapper fed alternative protein and lipid sources have been evaluated (Booth *et al.*, 2005; Booth *et al.*, 2012; Booth *et al.*, 2006; Glencross *et al.*, 2003b; Glencross *et al.*, 2003c; Glencross *et al.*, 2004c; Quartararo *et al.*, 1998a), in which the control diets contained a relatively large variety of protein and lipid levels (51 – 70 % and 6.8 – 17.5 %, respectively). The fishmeal can be substituted by 36 % poultry meal, or 35 % meat meal, and or 42 % soybean meal without impairing the growth performances and feed efficiency of snapper compared to those fed the commercial diet (Booth *et al.*, 2012). The small and large snapper also shows high capacity to digest gelatinized starch at inclusion levels less than 250 and 350 g/kg diet, respectively (Booth *et al.*, 2006), whereas fishmeal can be replaced up to 50 % by meat meal or poultry meal, and/or 30 % soybean meal without comprising the digestibility of nutrients (Booth *et al.*, 2005). When feeding to limited dietary protein, snapper fed Australian canola meal-based diet did not show any difference in growth and feed efficiency in comparison to those fed fishmeal-based diet (Glencross *et al.*, 2004d). Similar to fishmeal, some studies have indicated that up to 100 % of fish oil can be replaced by canola oil or soybean oil in

the diets for snapper without negative effects on growth, feed efficiency and proximate composition. The combination of 50 % soybean oil and 50 % fish oil can improve the final weight of snapper compared to those fed 100 % fish oil (Glencross *et al.*, 2003c). Although snapper fed different oil sources showed no difference in fatty acid profiles, however, fish fed fish oil diet as finisher diet after feeding with soybean oil or canola oil shows the increase in levels of long-chain polyunsaturated fatty acid (lcPUFA) such as 20: 5n-3 and 22: 6n-3 and decrease in PUFA such as 18: 2n-6 and 18: 2n-3 (Glencross *et al.*, 2003c).

2.4.4 Nutritional research on cobia

Carnivorous marine fish such as cobia requires high dietary protein to provide adequate amino acids and nitrogen for the synthesis of non-essential amino acids and other biological compounds (Fraser & Davies, 2009). The optimum dietary protein for maximal growth rate and feed utilisation in cobia is 44.5 % (Chou *et al.*, 2001), whereas Craig *et al.* (2006) reported that dietary protein levels (40 – 50 %) had no significant differences on nutritional responses of cobia (49.3 g/fish), but significantly affected the feed efficiency in smaller cobia (7.3 g/fish). The commercial feed for cobia in United States contains more than 58 % crude protein, while this level is less than 48 % in Taiwan and Asian countries (Craig *et al.*, 2006). The dietary methionine required for maximal SGR and FE in cobia is 1.19 % DM in the diet containing 0.67 % cysteine (Zhou *et al.*, 2006), whereas the optimum lysine requirement is 2.33 % DM (5.30 % of dietary protein) based on the maximum specific growth rate (SGR) (Zhou *et al.*, 2007). Ren *et al.* (2014) determined the arginine requirement in juvenile cobia to be 2.85 and 2.82% DM, respectively based on the maximal SGR and FE. Salze *et al.* (2012) indicated that dietary taurine supplementation improved the growth and activity of digestive enzymes in larval cobia, whereas juvenile cobia fed plant protein-based diets required taurine supplementation for potential growth (Lunger *et al.*, 2007).

According to Chou *et al.* (2001), dietary lipid required for cobia is 5.76% DM. Wang *et al.* (2005) found no significant differences in growth in cobia fed dietary lipid of 5 and 15 % DM, whereas the reduced growth was observed in cobia fed dietary lipid of 25 %. Craig *et al.* (2006) showed that dietary lipid level from 6 to 18 % has no impact on growth performance in small cobia (7.4 g/fish), but reduced growth was recorded in larger cobia (49.3 g/fish) fed dietary lipid of 18 %. The fatty acid requirements for larval cobia have been estimated based on the biochemical composition of eggs and yolksac

larvae (Sargent *et al.*, 1999). Fraser and Davies (2009) found high concentrations of highly unsaturated fatty acids (HUFAs) in the eggs and yolksac of cobia, which may indicate the high requirements for these fatty acids in cobia diets The dietary n-3 HUFA requirement for cobia broodstocks should be higher than 1.86 % DM, however, high dietary ARA (0.40 – 0.60 %) resulted negative effects on the fertilization of this species (Nguyen *et al.*, 2010).

Carbohydrates, a less costly energy source, is inefficiently utilised in marine carnivorous fish (de la Higuera, 2001). However, an adequate starch supplementation in diet can improve growth of some carnivorous fish (Hemre *et al.*, 2002). Ren *et al.* (2011) determined the optimum dietary carbohydrate requirement in juvenile cobia is 21.1 and 18.0 % based on the SGR and FER, respectively.

There is a little available information on the nutritional requirement of both vitamins and minerals in cobia, though, these elements play important roles for normal growth and physiological functions in fish (Fraser & Davies, 2009). Mai *et al.* (2009) determined the optimum dietary choline requirement to be 696, 877 and 950 mg/kg based on WG and tissue choline accumulations, respectively. The Se required for small cobia fed casein-based diet is 0.788, 0.811 and 0.793 mg/kg based on the SGR and Se concentrations in the tissues (Liu et al., 2010).

Fishmeal rather than terrestrial plant ingredients contains significantly higher protein content with well-balanced essential amino acids, high nutrient digestibility to meet nutritional requirement of the fish (Gatlin *et al.*, 2007). However, the rapid development of aquaculture has led to increased demand for fishmeal coupled with unstable supply (Olsen & Hasan, 2012; Tacon & Metian, 2008). Several studies have been evaluated to reduce the fishmeal dependence in cobia diets by using plant-derived ingredients for cobia (Chou *et al.*, 2004; Luo *et al.*, 2012; Romarheim *et al.*, 2008; Salze *et al.*, 2010; Suarez *et al.*, 2013; Zhou *et al.*, 2005). In soy products, 40% of protein from fishmeal can be substituted with soy-derived protein without comprising the growth performances and feed utilisation (Chou *et al.*, 2004; Zhou *et al.*, 2005), however, the optimum growth achieved at 16.9 and 18.9 % of replacement levels based on the quadratic regression. The reduced growth and increased feed conversion ratio (FCR) were reported when more than 40 % fishmeal protein were substituted with soybean meal. Whereas, 50 % and 94 % of fishmeal protein could be replaced with soy-derived protein and soy protein concentrate, respectively (Salze *et al.*, 2010; Trushenski *et al.*, 2011). Watson *et al.*

(2014b) demonstrated that non-genetically modified soybean could replace up to 70 % of protein in a referenced cobia diet without any negative effects on the growth and feed efficiency. Luo *et al.* (2012) determined the maximum dietary rapeseed meal in cobia diet to be 125 g/kg, whereas higher inclusion levels significantly reduced growth performance and digestibility of nutrients. Luo *et al.* (2013) showed that up to 300 g/kg corn gluten meal could be included in cobia diet without impairing the growth and feed intake (FI), while, dietary inclusion above this level resulted in reduced growth, FI and survival.

Organically certified yeast-based, another alternative protein ingredient has also been evaluated in juvenile cobia. The fish fed 195 g/kg yeast-based diet showed equal growth rate compared to those fed fishmeal-based diet. The increased plasma protein level also were observed in cobia fed diet containing 25 % yeast-based protein (Lunger *et al.*, 2006). The dietary taurine supplementation at 5 g/kg increased the inclusion level of yeast-based protein to be 40 % in cobia diet without impairing growth and feed efficiency (Lunger *et al.*, 2007). Besides, cobia fed diets containing 302 g/kg poultry-by product meal did not show any differences on growth, feed efficiency, proximate composition and haematological performance compared to those fed fishmeal-based diet (Zhou *et al.*, 2011).

Compared to fishmeal, plant-derived protein ingredients contain considerably amount of anti-nutritional factors such as saponins, phytate, tannins, oligosaccharides, alkaloids and protease inhibitors which can interfere the absorption and metabolism of nutrient components in fish (Francis *et al.*, 2001; Gatlin *et al.*, 2007). Moreover, the lacking of essential amino acids as methionine, lysine in plant meal can cause imbalanced amino acid profile when fishmeal is replaced with plant meal protein, consequently, resulting in reduced growth and feed efficiency, altering the histopathological performances in digestive tissues. The decreased growth and nutrient digestibility in cobia have been linked to the presence of high phytic acid and tannins concentrations in rapeseed meal (Luo *et al.*, 2012), whereas, Chou *et al.* (2004) showed that the methionine deficiency in soybean caused negative effects on growth and FCR in juvenile cobia.

To increase inclusion levels of plant-derived ingredients in aqua-feeds, the supplementation of amino acids, feed stimulants as flavourings and exogenous enzymes are necessary to enhance the performances of growth and feed utilisation (Gatlin *et al.*, 2007). The beneficial effects of trace element as selenium (Se) on growth, nutrient

digestibility and physiological responses in fish fed plant-based diets have been investigated in African catfish (Abdel-Tawwab *et al.*, 2007) and barramundi (Ilham *et al.*, 2016a). However, no published information is available on effects of dietary Se and plant-derived ingredients as lupin in carnivorous marine finfish such as Australian snapper and cobia.

CHAPTER 3. MATERIALS AND METHODS

A total of five feeding experiments were conducted during this study. Two different marine finfish species, snapper and cobia, showing extreme inherent growth rates, were selected to carry out these experiments. All feeding trials on snapper were conducted in the wet laboratory in Batavia Coast Maritime Institute (BCMI) located in Geraldton, Western Australia, Australia. Meanwhile experiments on cobia were carried out in the wet laboratory at Aquacultural Research Seed Production and Services Co., Ltd (ARSS) located in Nha Trang, Khanh Hoa, Vietnam.

3.1 EXPERIMENT 1: EFFECTS OF DIETARY SELENIUM SUPPLEMENTATION ON NUTRITIONAL AND PHYSIOLOGICAL RESPONSES OF SNAPPER

3.1.1 Experimental diets

The formulated basal diet (BS) contained different sources of protein including fishmeal, soybean meal and casein, while fish oil was used as the lipid source, was prepared. All ingredients used for the BS diet were obtained from the Specialty Feeds (Glen Forrest, Western Australia, 6071). The ingredients were finely ground and then mixed until homogenous before adding fish oil and distilled water. A commercial feed (CD), barramundi diet purchased from Skretting was used as referenced diet (NOVA ME 3.0 mm, Skretting, Tasmania, Australia).

Table 3.1.1 Ingredient composition of the formulated basal diet

Ingredients*	g/kg
Fishmeal	320
Soybean meal	100
Casein	146
Wheat flour	5
Wheat gluten	120
Cellulose	164
Wheat starch	30
Fish oil	95
Vitamin – Mineral premix[a] (Se free)	20

* *Supplied by Specialty Feeds (Glen Forrest, WA 6071, Australia).*

[a] *Contains the following (as g/kg 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; and ethoxyquin, 0.125.*

The commercial diet was ground to a fine powder, and 20 g/kg starch was added to the powder, and then thoroughly mixed with distilled water. The Se-yeast (0, 2 and 2.5 g) (Sel-Plex, Alltech, Nicholasville, KY, USA) was supplemented to both reconstituted commercial and formulated diets to provide 0; 0.8 and 1.0 mg Se/kg to produce 6 test diets labelled as CD0, CD0.8, CD1.0, BS0, BS0.8 and BS1.0, respectively. A mincer was used to pellet diet through 2 mm dye. The diets were air-dried, sealed in packed plastic bags and stored at -15 ^{0}C until feeding commenced. The diet formulation and proximate composition are presented in Table 3.1.1 and Table 3.1.2.

Table 3.1.2 Proximate compositions (dry weight basic) of the test diets

Diet	Crude protein (%)	Crude lipid (%)	Dry matter (%)	Ash (%)	Gross energy (MJ/kg)	Se content (mg/kg)	P/E	Carbohydrates (%)
CD0	49.52	16.20	92.88	9.60	21.64	0.92	2.29	24.68
CD0.8	49.30	15.58	92.82	9.41	21.58	1.68	2.28	25.71
CD1.0	49.52	16.32	92.98	9.36	21.90	1.76	2.26	24.80
BS0	46.56	11.50	93.45	8.80	20.70	1.67	2.25	33.14
BS0.8	46.54	11.35	92.41	8.60	21.02	2.59	2.21	33.51
BS1.0	46.36	11.45	93.12	8.83	21.13	2.77	2.19	33.36

Values are present as mean of three replicates. P/E: Crude protein to gross energy ratio.

3.1.2 Fish rearing

Juvenile snapper were obtained from the BCMI. The fish were kept in a 4 m^3 fibreglass tank and fed commercial barramundi diet (NOVA ME 3.0 mm, Skretting, Tasmania, Australia) containing 50 % crude protein, 16 % lipid and 21 MJ/kg of gross energy. Before the feeding experiment commenced, all fish were starved for 24 hours and anaesthetised with AQUI-S (AQUI-S New Zealand Ltd, New Zealand), individually weighed and measured for their individual standard lengths. The snapper (initial mean weight, 142.47 g/fish) were randomly distributed to eighteen 150 L-fiberglass tanks with ten fish per tank. Each experimental diet was assigned to three randomly positioned tanks. All tanks were connected with the seawater supply to maintain the water flow rate at approximately 5 L/min. Air diffuser and oxygen were used to keep the oxygen no less than 5.5 mg/L. The temperature, oxygen and pH in the rearing water were measured daily using OxyGuard meters (Handy Polaris 2 and Handy pH, OxyGuard International A/S, Denmark). Total ammonia was daily monitored with the NH_3/NH_4^+ test kit (Mars Fishcare, Chalfont, PA, USA). The fish were hand-fed twice daily at 8:00 and 16:00 until apparent satiation for ten weeks. After feeding, the uneaten feed was obtained

through 150 µm net and rinsed by fresh water, and stored at – 15 ^{0}C for further analysis. During the entire feeding trial, ammonia was less than 0.25 mg/L, while average pH, dissolved oxygen and water temperature were 7.6 ± 0.2, 6.5 ± 0.3 mg/L and 24.7 ± 0.7 ^{0}C, respectively.

3.1.3 Sample collection

At the beginning of the feeding trial, five fish were randomly selected to analyse initial whole body composition. After ten weeks of the experiment, all fish were individually weighed and measured for their body lengths. Before weighing them individually, the fish were starved for 24 hours and anaesthetised with AQUI-S. Three fish in each tank were collected to determine body composition. Another three fish were used to remove liver, and muscle tissues for proximate composition and selenium analysis. The tissues were cut before fixing in 10% buffered formalin for histopathological evaluation.

3.1.4 Chemical analysis

Crude protein content was analysed using Kjeltec Auto 1030 analyser (Foss Tecator, Höganäs, Sweden). Crude fat was analysed by Soxhlet technique using Soxtec System HT6 (Tecator, Höganäs, Sweden). Moisture was determined by drying at 105 ^{0}C in an oven (Thermotec 2000, Contherm Scientific, Hutt, New Zealand). Ash was determined by combustion at 550 ^{0}C for 24 h in an electric furnace (Carbolite, Sheffield, UK). Gross energy was determined by bomb calorimeter (C2000, IKA, Staufen, Germany).

For the Se analysis, samples of liver, muscle and diets were digested in digestion solution (the mixture of HNO_3 and $HCIO_4$ at 10:3 ratio) using block digestion system (AIM 500-C, A.I. Scientific, Sydney, NSW, Australia). Then, acid hydrochloric (40%) was used further to extract the digested solution for conversion of Se^{6+} to Se^{4+}. The Se level was estimated following AOAC (1990) using an atomic absorption spectrometer (Varian AA280 FS and Varian VGA 77, Mulgrave, VIC, Australia).

3.1.5 Histological examination

For histological examination, the liver samples were subsequently dehydrated in ethanol. After equilibration in xylene, samples were embedded in paraffin. Five sections from each specimen of the liver with approximately 5 µm were sliced before staining in the solution of haematoxylin and eosin (H&E). The sections of the samples were observed

under light microscopy (ECLIPSE 80i, Nikon, Japan) with 200 x and 400 x magnification.

3.1.6 Calculations and statistical analysis

The following parameters in all tanks were calculated using the following equations:

Survival (%) = 100 x final fish number / initial fish number

Weight gain (WG g/fish) = final weight – initial weight

Daily Growth Coefficient (DGC %/day) = 100 x ($FBW^{1/3}$ – $IBW^{1/3}$)/days

Feed Conversion Ratio (FCR) = feed intake in dry matter (g)/body weight gain (g)

Protein Efficiency Ratio (PER) = body weight gain (g)/protein fed (g)

Energy Efficiency Ratio (EER) = body weight gain (g)/ energy fed

Protein retention (PR) = protein gain x 100/protein fed

Energy retention (ER) = energy gain x 100/energy fed

Hepatosomatic Index (HSI) = 100 x liver weight/whole body weight

Viscerasomatic Index (VSI) = 100 x viscera weight/whole body weight

Condition Factor (CF %) = 100 x live weight (g)/(body length cm)^3

Coefficient of Variances (CV %) = 100 x standard deviation/mean

Thermal growth coefficient (TGC) (Bureau *et al.*, 2002)

TGC = ($FBW^{1/3}$- $IBW^{1/3}$)/(temperature ^{0}C x days) x 1000

All data were statistically analysed using SPSS for Windows version 22 (IBM, New York, USA) unless otherwise specified. The Shapiro-Wilk and Levene's tests were performed to test the normality and homogeneity of variance of all data. Data on survival was arcsine transformed before analysis. The two-way ANOVA was used to analyse the effects of diet types, Se supplementation and their interaction on all of the tested parameters of snapper. When a significant main effect was observed, data were analysed with one-way ANOVA followed by Turkey's post hoc test to determine the differences among the means of main effects. When a significant interaction was detected, one-way analysis (ANOVA) with post hoc Turkey's HSD multiple comparison tests were employed to determine differences among all dietary treatments, but not for means of

main effects. The linear regression analysis was used to determine the relation among dietary Se level with Se accumulation in the tissues. The significance level was $P < 0.05$.

3.2 EXPERIMENT 2: NUTRITIONAL ROLES OF SELENIUM SUPPLEMENTATION ON GROWTH AND PHYSIOLOGICAL PERFORMANCES OF COBIA

3.2.1 Experimental diet

A commercial cobia diet (NANOLIS C2, Guyomarc'h, Vietnam) containing fishmeal, soybean meal and wheat flour as protein sources was selected to produce the test diets. This feed contains 52.78 % protein, 8.85 % lipid and 21.53 MJ/kg gross energy. The Se-yeast (Sel-Plex, Alltech, Nicholasville, KY, USA) was top coated to the test diets using gelatin (Davis Gelatine, Christchurch, New Zealand) following the method described by Le and Fotedar (2013) to provide 6 Se supplemented levels of 0 (control diet), 0.4, 0.8, 1.2, 1.6 and 2.0 mg/kg to produce 6 test diets. The actual Se levels in test diets were 1.15, 1.52, 1.91, 2.29, 2.71 and 3.14 mg/kg, respectively.

3.2.2 Fish rearing

Juvenile cobia hatched and reared at the ARSS Company was used for this trial. Fish were fed the control diet for two weeks to acclimate to experimental conditions. Before feeding commenced, all fish were starved for 24 hours and individually weighed after being anaesthetised with AQUI-S (AQUI-S New Zealand Ltd, New Zealand) at 10 ml/1000 L. The cobia (average weight 13.65 ± 0.40 g/fish) were randomly distributed to eighteen tanks (500 L/tank) at a density of 12 fish per tank. Each experimental diet was assigned to three randomly positioned tanks. A seawater source containing less than 1 µg/L Se was supplied to maintain the water flow rate at approximately 5 L/min. Fish were hand-fed twice daily at 8:00 and 16:00 until apparent satiation for 8 weeks. Water temperature, dissolved oxygen and pH were measured daily using OxyGuard meters (Handy Polaris 2 and Handy pH, OxyGuard International A/S, Denmark). Total ammonia was daily monitored with NH_3/NH_4^+ test kit. Throughout the feeding trial, the temperature ranged from 27.5 to 29.3 ^{0}C, dissolved oxygen ranged from 6.2 -6.8 mg/L, total ammonia was less than 0.50 mg/L, while pH was 7.5- 8.1. The salinity ranged from 29 - 33 ‰.

3.2.3 Sample collection

Fifteen juveniles were randomly collected for initial whole-body composition analysis at the beginning of the feeding trial. At the end of the feeding trial, all fish were individually weighed after 24 hours of starvation. Blood samples from three fish per tank were collected by puncturing their caudal vein using a syringe and transferred to BD Vacutainer (with K2E 5.4 mg, United Kingdom) for haematological analysis. The remaining blood was transferred into Eppendorf tubes and allowed to clot in a fridge at 4 ^{0} C for 2 hours before collecting red blood cells by centrifuging whole blood at 5000 rpm in 10 mins. The pellets of red blood cell (RBCs) were stored in a freezer for glutathione peroxidase (GPx) analysis. Three fish in each tank were collected to analyse body composition. Liver and flesh tissues from six fish were dissected for proximate composition and selenium analysis. The distal intestine and livers from three fish in each tank were dissected out before fixing in 10% buffered formalin for histopathological evaluation.

3.2.4 Chemical, haematological and enzymatic analysis

The proximate composition and selenium concentrations were analysed according to the methods mentioned in section 3.1.4. Blood constituents of cobia were automatically determined using automated hematology analyser (Sysmex XT-162 1800i, Kobe, Japan). GPx in RBCs was analysed by the Ransel reagent (Randox, Antrim, United Kingdom). The selenium concentration in the rearing water, cobia muscles and the test diets samples were analysed following the method described in section 3.1.4.

3.2.5 Histological evaluation

The histopathological performance was analysed following the method described in section 3.1.5. The sections of the samples were examined by light microscopy (Olympus BX51, Tokyo, Japan) with 400 x magnification.

3.2.6 Statistical analysis

The variables in all tanks were calculated using equations described in section 3.1.6.

Specific Growth Rate (SGR %/day) = 100 x [(Ln FBW – Ln IBW)/feeding period (days)]

Mean corpuscular volume (MCV) = Haematocrit/Red blood cell

Mean corpuscular haemoglobin concentration (MCHC) = Haemoglobin/Haematocrit

All data were expressed as mean ± SE unless otherwise specified and analysed by one-way ANOVA. The normality and homogeneity of variance of all data was tested with he Shapiro-Wilk and Levene's tests. Data on survival was arcsine transformed before analysis. To analyse the significant differences in feeding treatments, Turkey's HSD multiple comparison post hoc tests were employed using SPSS for Windows version 22 (IBM, New York, USA). The linear regression analysis was applied to determine the relationship between dietary Se levels and tissue Se accumulations. The second-degree polynomial regression analysis was used to perform the SGR against the dietary Se level. The significant statistic was evaluated at $P < 0.05$.

3.3 EXPERIMENT 3: NUTRITIONAL AND PHYSIOLOGICAL RESPONSES OF SNAPPER FED VARIOUS DIETARY LUPIN KERNEL MEAL INCLUSIONS

3.3.1 Experimental diet preparation

Five isonitrogenous and isoenergetic experimental diets were formulated to contain approximately 46 % crude protein and 11 % crude lipid in which 0% (control; LP0); 20% (LP20); 40% (LP40); 60% (LP60) and 80% (LP80) of the fishmeal protein was substituted by narrow-leafed lupin kernel meal (LKM) protein. All ingredients were obtained from the Specialty Feeds (Glen Forrest, Western Australia 6071, Australia).

Table 3.3.1 Feed ingredient composition (g/100 g or MJ/kg in dry matter)

Ingredients [a]	Crude protein	Total lipid	Ash	Dry matter	Organic matter [b]	Gross energy
Fishmeal	64.00	10.70	21.90	91.43	78.10	20.09
Soybean meal	47.81	0.70	6.77	88.91	93.23	20.09
Lupin meal	39.00	6.82	2.41	87.61	97.59	20.93
Casein	88.88	0.91	1.07	85.04	98.93	24.78
Wheat flour	11.96	1.51	0.31	83.89	99.69	18.72
Wheat gluten	75.85	0.53	0.25	91.47	99.75	23.87
Starch	N/A	N/A	0.30	86.60	99.7	17.74
Fish oil	N/A	99.51	0.28	99.82	99.72	43.00

[a] *Purchased from Specialty Feeds (Glen Forrest, WA 6071, Australia).*
[b] *Calculated by difference = (100 – measured ash content)*

The ingredients were finely ground, and then homogenously mixed. Chromic oxide (Cr_2O_3) was added to all tested diets as an inner marker. Fish oil and distilled water were

added and thoroughly mixed. A laboratory extruder was used to pelletise the diet through a 2 mm die. The diets were air-dried to approximately 90 g/kg moisture, sealed in packed bags and stored at -15^{0}C until feeding trials commenced. The ingredient and diet compositions are presented in Table 3.3.1 and Table 3.3.2.

Table 3.3.2 Ingredients and composition of the experimental diets g/kg in dry weight)

Ingredients [a]	Diet groups				
	LKM0	LKM105	LKM210	LKM315	LKM420
Fishmeal	320	256	192	128	64
Soybean meal	100	100	100	100	100
Lupin kernel meal	0	105	210	315	420
Casein	146	146	146	146	146
Wheat flour	5	5	5	5	5
Wheat gluten	120	120	120	120	120
Cellulose	164	123	82	41	0
Wheat starch	30	30	30	30	30
Fish oil	95	95	95	95	95
Premix[b]	15	15	15	15	15
Chromic oxide[c]	5	5	5	5	5
Proximate composition (% dry matter)					
Moisture (%)	8.82	8.66	10.29	11.79	9.56
Crude protein (%)	46.56	46.64	46.85	46.24	46.71
Crude lipid (%)	11.42	11.94	11.35	11.07	10.97
Ash (%)	8.10	7.09	6.46	5.72	4.53
Gross energy (MJ/kg)	20.99	21.71	22.15	22.34	22.80
Amino acids (g/100 g dry sample)					
Arginine	2.77	2.81	3.15	3.37	3.50
Histidine	1.46	1.31	1.39	1.35	1.29
Isoleucine	2.27	2.19	2.33	2.36	2.30
Leucine	4.32	4.00	4.20	4.21	4.11
Lysine	3.08	2.55	2.80	2.65	2.44
Methionine	1.31	1.14	1.10	1.03	0.93
Phenylalanine	2.41	2.27	2.40	2.42	2.37
Threonine	2.25	2.07	2.12	2.10	2.03
Valine	2.63	2.50	2.62	2.63	2.57
Taurine	0.05	0.03	0.03	0.00	0.00
Lysine/Arginine	1.11	0.91	0.89	0.79	0.70

[a] *Purchased from Specialty Feeds (Glen Forrest, WA 6071, Australia).*

[b] *Contains the following (as g/kg 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; and ethoxyquin, 0.125.*
[c] *Purchased from Thermo Fisher Scientific, Scoresby, Victoria, Australia.*

3.3.2 Fish rearing

Juvenile snapper hatched and reared at the Batavia Coast Maritime Institute was used in this study. Before the beginning of the feeding trial, snapper was acclimated to the experimental conditions for 2 weeks. During this period, snapper were fed a commercial barramundi diet (NOVA ME, Skretting, Tasmania, Australia) containing 50 % protein, 17 % lipid and 21.1 MJ/kg gross energy. Before commencing feed trial, all fish were anaesthetised by AQUI-S (AQUI-S New Zealand Ltd, New Zealand) at 10 ml/1000L and individually weighed. The snapper (initial mean weight of 21.64 g) were randomly distributed to fifteen 150L-fiberglass tanks with stocking density of 12 fish per tank. Each experimental diet was assigned to three randomly positioned tanks. All experimental tanks were connected to a continuously flow-through seawater supply to maintain the water flow rate at approximately 5 L/min. The fish were hand-fed twice daily at 8:00 and 16:00 until apparent satiation. The feeding experiment was conducted for 70 days. After 10 minutes of feeding, uneaten feed was siphoned through a 150 µm net and rinsed with fresh water, and then stored in freezers until analysis. The temperature, salinity and dissolved oxygen were monitored daily, whereas pH and total ammonia were monitored once per week. The temperature in the rearing water ranged from 19.2 to 22.5 ^{0}C, total ammonia was less than 0.25 mg/L, and pH was 7.5- 8.0. The salinity in rearing systems ranged from 28 - 33 ppt.

3.3.3 Sample collection

Fifteen fish were randomly sampled for an analysis of their initial whole body composition. At the end of feeding trial, fish were anaesthetised by AQUI-S at 15 mg/L and individually weighed after starving for 24 hours. Three fish per tank were sampled to analyse body composition. Another three fish were used to collect blood samples by puncturing their caudal vein with a syringe and transferred to BD Vacutainer (with K2E 5.4 mg, UK) for haematological analysis. The liver and muscle tissues from six fish per tank were collected for proximate composition analysis. The distal intestines and livers of snapper were cut and fixed in Bouin solution for histopathological evaluation. Faeces were collected from week 6 of the feeding period according to procedures of Kim *et al.*

(2007) until an adequate amount for faeces was available for the digestibility analysis. One hour after the last feeding, the air diffusers, and the bottom and walls of the tanks were carefully cleaned to remove any residual feed and waste from the rearing system. Faeces were allowed to settle overnight. The faeces were gently collected into a 200 mL polyethylene bottle in the following morning and then were centrifuged for 20 min at 10000 rpm before the supernatant was discarded; the faecal material was stored at -20 ^{0}C until analysis.

3.3.4 Chemical analysis

Proximate compositions in the samples were analysed following the protocols mentioned in section 3.1.4. Amino acid compositions in the muscle and test diets were analysed using high performance liquid chromatography after acid hydrolysis. Cr_2O_3 was determined by the method from Bolin *et al.* (1952).

3.3.5 Haematological examination

Blood parameters in snapper were analysed following protocols:

The Ht was determined following method described by Rey Vázquez and Guerrero (2007). Blood was collected into heparin-coated microhaematocrit tubes (Livingstone, Rosebery, NSW, Australia) before centrifuging at 13000×*g* for 5 min. Ht level was measured as the percentage of packed cell volume.

The RBC was determined following method described by Le *et al.* (2014a). Microscope slides were used to make blood smear. After staining with May-Grünwald and Giemsa solutions, the total RBC was calculated using a light microscope (ECLIPSE 80i, Nikon, Japan) and the relative percentage was calculated. A haemocytometer was used to count total number of blood cells. The RBC value was calculated by multiplying relative percentage with total blood cells.

The Hb was measured using the Hb HG-1539 kit (Randox, Crumlin, County Antrim, UK) and a chemistry immune analyser (Olympus AU400, Tokyo, Japan).

3.3.6 Histological evaluation

The histopathological performance was analysed following the method described in section 3.1.5. The sections of the samples were examined by light microscopy (Olympus BX51, Tokyo, Japan) with 400 x magnification.

3.3.7 Statistical analysis

The variables in all tanks were calculated using the equations described in section 3.1.6 and following formula:

Apparent digestibility coefficients (ADCs) of tested diets and the basal diet were calculated using the formula described by Cho *et al.* (1982):

$ADC_{diet} = 100 \times [1-(F_{nut}/D_{nut} \times D_{Cr}/F_{Cr})]$

where F_{nut} is the % of nutrient or gross energy in faeces, D_{nut} represents % of nutrient or gross energy in the diet, and D_{Cr} and F_{Cr} are % of the chromic oxide in diet and faeces, respectively.

The ADC of ingredients in each diet were calculated following the equation from Sugiura *et al.* (1998):

$ADC_{ingredient} = [(Nut_{TD} \times ADC_{TD}) - (Nut_{BS} \times ADC_{BS} \times P_{BS})]/(P_{Ingredient} \times Nut_{Ingredient})$

where Nut_{TD}, Nut_{BS} and $Nut_{ingredient}$ represent for % of the nutrient or gross energy in the tested diet, basal diet and ingredient, respectively. ADC_{TD} and ADC_{BS} are % of the apparent digestibility coefficient of nutrient or gross energy in the tested and basal diet, respectively. P_{BS} is the proportion of the basal diet, and $P_{Ingredient}$ is the proportional amount of ingredient in tested diet.

All data were expressed as mean ± SE unless otherwise specified and analysed by one-way analysis of variance (ANOVA). The Shapiro-Wilk and Levene's tests were used examine the normality and homogeneity of variance. Data on survival was arcsine transformed before analysis. To analyse the significant differences in feeding treatments, Turkey's HSD multiple comparison post hoc tests were employed using SPSS for Windows version 22 (IBM, New York, USA). The linear regression model was applied to evaluate the relationship between dietary lupin kernel meal inclusions and the SGR, FCR and PER of snapper.

3.4 EXPERIMENT 4: NUTRITIONAL AND PHYSIOLOGICAL RESPONSES OF COBIA FED DIETARY LUPIN KERNEL MEAL LEVELS

3.4.1 Experimental diet preparation

The experimental diets used in this feeding trial were similar to test diets previously described in section 3.3.1.

3.4.2 Fish rearing

Juvenile cobia hatched and reared at the ARSS Company (Khanh Hoa Province, Vietnam) was used in this experiment. Before the commencement of feeding trial, cobia were acclimated to the experimental conditions for 2 weeks using a commercial cobia diet (NRD P16, INVE Ltd, Thailand) containing 53 % protein, 12 % lipid and 21.0 MJ/kg gross energy. Prior to the test feeding, all fish were starved for 24 hours and anaesthetised by AQUI-S (AQUI-S New Zealand Ltd, New Zealand) at 10 mg/L and individually weighed. The cobia (initial mean weight of 18.05 g) were randomly distributed to fifteen tanks (500 L) at a density of 12 fish per tank. Each experimental diet was assigned to three randomly positioned tanks. All experimental tanks were connected to a continuously flow-through seawater supply to maintain the water flow rate at approximately 5 L/min. The fish were hand-fed twice daily at 8:00 and 16:00 until apparent satiation. The feeding trial lasted for 56 days. After 10 minutes of feeding, uneaten feed was siphoned through a 150 µm net and rinsed with fresh water, and then stored in freezers until analysis. The water parameters were measured following methods in section 3.2.2. During the feeding trial, the water temperature ranged from 28 - 30 ^{0}C, total ammonia was less than 0.25 mg/L, and pH was 7.8- 8.2. The salinity in rearing systems ranged from 28 - 33 ppt.

3.4.3 Sample collection

Sample collection was similar to those described in section 3.3.3.

3.4.4 Chemical analysis

Proximate compositions in the samples were analysed following the protocols mentioned in section 3.1.4. Blood constituents of cobia were automatically determined using automated hematology analyser (Sysmex XT-162 1800i, Kobe, Japan). Amino acid compositions in the muscle and test diets were analysed using high performance liquid

chromatography after acid hydrolysis. Chromic oxide was determined by the method from Bolin *et al.* (1952).

3.4.5 Histological evaluation

The histopathological performance was analysed following the method described in section 3.3.6.

3.4.6 Calculations and statistical analysis

The variables in all tanks and the digestibility were calculated using the equations in section 3.1.6 and 3.3.7.

All data were expressed as mean ± SE unless otherwise specified and analysed by one-way analysis of variance (ANOVA). The Shapiro-Wilk and Levene's tests were performed to test the normality and homogeneity of variance of all data. Data on survival was arcsine transformed before analysis. To analyse the significant differences in feeding treatments, Turkey's HSD multiple comparison post hoc tests were employed using SPSS for Windows version 22 (IBM, New York, USA). The linear regression model was applied to evaluate the relationship between dietary lupin kernel meal inclusions and the SGR, FCR and PER of cobia. The differences between snapper in the section 3.3 and cobia in section 3.4 in all of the tested parameters at each inclusion level of lupin kernel meal in the third and fourth experiments were analysed using T-test: two samples. The difference between the correlation coefficients (R-squared values) of the SGR, FCR and PER of snapper and cobia also were identified. The statistical significance was evaluated at $P < 0.05$.

3.5 EXPERIMENT 5: BENEFICIAL EFFECTS OF SELENIUM SUPPLEMENTATION ON COBIA FED LUPIN-BASED DIETS

3.5.1 Experimental diet preparation

The fishmeal protein in the control diet (LP0) was replaced with 40% and 60% of narrow-leafed lupin kernel meal (LKM) protein with and without 0.8 mg/kg Se extracted from Se-yeast (Sel-Plex, Alltech, Nicholasville, KY, USA) supplementation to formulate six isonitroenous and isoenergetic test diets, labelled as LP0, LP0Se, LP40, LP40Se, LP60 and LP60Se, respectively. The ingredients were finely ground, and then homogenously mixed. Chromic oxide (Cr_2O_3) was added to all tested diets as inner

marker. Taurine was added to all diets at 0.5 g/kg to meet the nutritional requirement of cobia. Fish oil and distilled water were added and thoroughly mixed. A laboratory extruder was used to pellet diet through 2 mm die. After drying, pellets were sealed in packed bags, and then stored in a freezer until feeding trial commenced. The diet formulation, compositions and amino acid profiles are presented in Table 3.5.1, Table 3.5.2 and Table 3.5.3.

Table 3.5.1 Ingredients and composition of the experimental diets (g/kg in dry weight)

Ingredients[a]	Diet group					
	LP0	LP0Se	LP40	LP40Se	LP60	LP60Se
Fishmeal	320	320	192	192	128	128
Soybean meal	100	100	100	100	100	100
Lupin kernel meal	0	0	210	210	315	315
Casein	146	146	146	146	146	146
Wheat flour	4.5	4.5	4.5	4.5	4.5	4.5
Wheat gluten	120	120	120	120	120	120
Cellulose	164	164	82	82	41	41
Wheat starch	30	29	30	29	30	29
Fish oil	95	95	95	95	95	95
Taurine	0.5	0.5	0.5	0.5	0.5	0.5
Premix	15	15	15	15	15	15
Sel-Plex[c]	0	1.5	0	1.5	0	1.5
Chromic oxide[d]	5	5	5	5	5	5
Proximate composition						
Moisture (%)	8.82	8.66	10.29	11.79	9.56	10.02
Crude protein (%)	46.51	46.64	46.85	46.24	46.71	46.54
Crude lipid (%)	11.42	11.94	11.35	11.07	10.97	10.12
Ash (%)	8.10	7.09	6.46	5.72	4.53	4.58
Gross energy (MJ/kg)	20.85	20.78	21.38	21.36	22.18	22.21
Se (mg/kg)	1.71	2.42	1.28	2.04	1.03	1.82
Phytic acid (g/kg)	2.51	2.48	3.58	3.61	3.94	4.01
Tannins (g/kg)	0.35	0.33	0.61	0.63	0.91	0.90

[a] *Supplied by Specialty Feeds (Glen Forrest, WA 6071, Australia).*
[b] *Contains the following (as g/kg 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; and ethoxyquin, 0.125.*
[c] *Obtained from Alltech, Nicholasville, KY, USA.*
[d] *Obtained from Thermo Fisher Scientific, Scoresby, Victoria, Australia.*

3.5.2 Fish rearing

Juvenile cobia hatched and reared at the ARSS Company was used in this experiment. Before the beginning of the feeding trial, cobia were fed a commercial cobia diet (NRD P16, INVE Ltd, Thailand) containing 53 % protein, 12 % lipid and 21.0 MJ/kg gross energy in 2 weeks for acclimation with experimental conditions. Before feeding commencement, all fish were individually weighed after starving for 24 hours and anaesthetisation with AQUI-S (AQUI-S NewZealand Ltd, New Zealand). The cobia (initial mean weight of 18.56 g/fish) were randomly distributed to eighteen 500-L fiberglass tanks at a density of 12 fish per tank. Each test diet was assigned to three randomly positioned tanks. All experimental tanks were connected to a continuously flow-through seawater supply to maintain the water flow rate at approximately 5 L/min. Fish were hand-fed twice daily at 8:00 and 16:00 until apparent satiation for 7 weeks. After 10 minutes of feeding, uneaten feed was siphoned through a 150 µm net and rinsed with fresh water, and then stored in freezers until analysis. The temperature, salinity and dissolved oxygen were daily monitored, whereas pH, total ammonia were monitored once per week. During the feeding trial, the water temperature ranged from 28 - 30 ^{0}C, total ammonia was less than 0.5 mg/L, while pH was 7.6- 8.2. The salinity ranged from 28 - 33 ‰.

Table 3.5.2 Feed ingredient composition (g/100 g or MJ/kg in dry matter) unless specified

Ingredients [a]	Crude protein	Total lipid	Ash	Dry matter	Organic matter [b]	Gross energy	Se mg/kg	Phytic acid	Tannins
Fishmeal	64.00	10.70	21.90	91.43	78.10	20.09	4.37	n/a	n/a
Lupin meal	39.00	6.82	2.41	87.61	97.59	20.93	0.35	0.53	0.16

n/a: not analysed.

3.5.3 Sample collection

Fifteen fish were randomly sampled for an analysis of their initial whole body composition. At the termination of the feeding trial, three fish per tank were randomly sampled to analyse body composition. The muscle tissues from four fish in each tank were pooled and used for chemical composition, selenium content and amino acid analysis. Another three fish were used to collect blood samples by puncturing their caudal vein using a needle and syringe and transferred to BD Vacutainer (with K2E 5.4 mg, UK) for haematological analysis. Faeces were collected following the method described in section 3.3.3.

Table 3.5.3 Essential amino acid profiles of the experimental diets (g/100g dry sample)

Ingredients[a]	Diets					
	LP0	LP0Se	LP40	LP40Se	LP60	LP60Se
Essential amino acids						
Arginine	2.78	2.78	3.17	3.17	3.37	3.36
Histidine	1.45	1.46	1.39	1.38	1.34	1.35
Isoleucine	2.29	2.27	2.31	2.31	2.36	2.37
Leucine	4.31	4.34	4.22	4.21	4.18	4.20
Lysine	3.06	3.06	2.81	2.80	2.61	2.59
Methionine	1.33	1.31	1.14	1.13	1.05	1.06
Phenylalanine	2.41	2.42	2.38	2.40	2.40	2.41
Threonine	2.26	2.26	2.14	2.15	2.13	2.12
Valine	2.60	2.61	2.61	2.62	2.63	2.63
Taurine	0.42	0.41	0.43	0.41	0.39	0.40

3.5.4 Chemical and haematological analysis

Proximate composition were analysed following the methods mentioned in section 3.1.4. Blood constituents of cobia were automatically determined using automated hematology analyser (Sysmex XT-162 1800i, Kobe, Japan). Selenium contents in the tissues and diets were determined following method described in section 3.1.4. Amino acid compositions in the muscle and test diets were analysed by using high performance liquid chromatography after acid hydrolysis. Chromic oxide was determined by the method from Bolin *et al.* (1952). The tannin was analysed following method described by Embaby (2011) after extraction in acetone. Phytic acid in phytate salt form was analysed after extraction in hydrochloric acid following procedure from Haddad *et al.* (2007).

3.5.5 Statistical analysis

The variables in all tanks were calculated using the equations described in section 3.1.6. Apparent digestibility coefficient (ADC) of tested diets and the basal diet were calculated using the formula described by Cho *et al.* (1982).

$$ADC_{diet} = 100 \times [1-(F_{nut}/D_{nut} \times D_{Cr}/F_{Cr})]$$

where F_{nut} is the % of nutrient or gross energy in faeces, D_{nut} represents % of nutrient or gross energy in the diet, and D_{Cr} and F_{Cr} are % of the chromic oxide in diet and faeces, respectively.

All data were statistically analysed using SPSS for Windows version 22 (IBM, New York, USA) unless otherwise specified. Data on survival was arcsine transformed before analysis. The two-way ANOVA was used to analyse the effects of lupin meal inclusions, Se supplementation and their interactions on all of the tested parameters of cobia. When a significant main effect was observed, data were analysed to determine the differences among the dietary groups with or without Se supplementation. When a significant interaction was detected, one-way analysis (ANOVA) with post hoc Turkey's HSD multiple comparison tests were employed to determine differences among dietary treatments, but not for means of main effects. The linear regression analysis was used to determine the relationship between dietary Se level and Se accumulation in the tissues. The statistical significance was evaluated at $P < 0.05$.

CHAPTER 4. RESULTS

4.1 EXPERIMENT 1: EFFECTS OF DIETARY SELENIUM SUPPLEMENTATION ON NUTRITIONAL AND PHYSIOLOGICAL RESPONSES OF SNAPPER

4.1.1 Growth performance and feed utilisation

Growth performance and survival of snapper fed dietary Se supplemented diets are displayed in Table 4.1.1. All snapper accepted the experimental diets and after 10 weeks of feeding, there was no effect ($P > 0.05$) of diet types, Se supplementation and their interaction on the survival rate of the juvenile fish. The final body weight (FBW), weight gain (WG) and daily growth coefficient (DGC) were not significantly ($P > 0.05$) affected by the diet types and the interaction between diet and Se supplementation.

Table 4.1.1 Growth performances of Australian snapper fed diets with Se supplementation

	IBW (g/fish)	FBW (g/fish)	DGC (%/day)	WG (g)	Survival (%)
Diets					
CD0	141.95	225.80	1.25	83.85	100
CD0.8	142.34	234.66	1.35	92.32	100
CD1.0	141.82	208.57	1.02	66.74	100
BS0	142.26	229.71	1.29	87.46	100
BS0.8	142.09	229.15	1.29	87.06	100
BS1.0	142.16	202.79	0.94	60.64	96.67
S.E.M	0.199	3.21	0.04	3.14	0.001
Means of main effects of diet types					
CD	142.04	223.01	1.21	80.97	100
BS	142.17	220.55	1.17	78.38	99.63
Means of main effects of Se					
0	142.11	227.76^{y}	1.27^{y}	85.65^{y}	100
0.8	142.22	231.90^{y}	1.32^{y}	89.69^{y}	100
1.0	141.99	205.68^{x}	0.98^{x}	63.69^{x}	99.45
Two-ways ANOVA: P-values					
Diet types	0.785	0.490	0.419	0.432	0.337
Se	0.923	0.000	0.000	0.000	0.397
Diet types x Se	0.845	0.452	0.435	0.413	0.397

Values are displayed as mean of triplicate groups. Means with different alphabets (x, y) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of dietary Se supplementation. IBW Initial body weight; FBW Final body weight; DGC Daily growth coefficient; S.E.M Standard error of means; Se Selenium; CD Reconstituted commercial diet; BS Basal diet.

Dietary Se supplementation significantly ($P < 0.05$) affected the FBW and DGC of snapper, where fish fed diet supplemented with 1.0 mg/kg Se resulted in the significantly ($P < 0.05$) lower FBW, WG and DGC than the fish fed lower Se-supplemented diets. Regardless of the diet types, dietary Se supplementation of 0.8 mg/kg did not alter the FBW, WG and DGC of the snapper.

Table 4.1.2 Feed utilisation of Australian snapper fed diets with Se supplementation

	FI (g/fish)	FCR	PER (%)	EER (%)	PR (%)	ER (%)
Diets						
CD0	113.39	1.35	1.49	3.41	27.35	30.83
CD0.8	124.86	1.36	1.49	3.42	27.43	37.26
CD1.0	108.76	1.66	1.22	2.75	23.06	26.88
BS0	96.53	1.10	1.95	4.30	36.30	38.33
BS0.8	93.88	1.08	2.01	4.44	39.32	42.58
BS1.0	78.45	1.31	1.67	3.66	27.30	26.01
S.E.M	4.43	0.05	0.07	0.15	1.56	1.77
Means of main effects of diet types						
CD	115.67^{b}	1.45^{b}	1.40^{a}	3.19^{a}	25.95^{a}	31.66
BS	89.62^{a}	1.16^{a}	1.87^{b}	4.14^{b}	34.31^{b}	35.64
Means of main effects of Se						
0	104.97	1.23^{x}	1.72^{y}	3.86^{y}	31.83^{y}	34.58^{y}
0.8	109.37	1.22^{x}	1.75^{y}	3.93^{y}	33.38^{y}	39.92^{y}
1.0	93.61	1.48^{y}	1.44^{x}	3.21^{x}	25.18^{x}	26.44^{x}
Two-ways ANOVA: P-values						
Diet types	0.001	0.000	0.000	0.000	0.000	0.107
Se	0.130	0.001	0.004	0.003	0.005	0.001
Diet types X Se	0.573	0.702	0.914	0.928	0.225	0.334

Values are displayed as mean of triplicate groups. Means with different alphabets (a, b or x, y) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of diet types and dietary Se supplementation, respectively. FI Feed intake; FCR Feed conversion ratio; PER Protein efficient ratio; EER Energy efficient ratio; PR Protein retention; ER Energy retention; S.E.M Standard error of means; Se Selenium; CD Reconstituted commercial diet; BS Basal diet.

The feed utilisation efficiency of snapper fed different supplemented Se diets is presented in Table 4.1.2. There were no significant ($P > 0.05$) effects of dietary Se supplementation and the interaction between diet types and dietary Se supplementation on the feed take (FI), but was significantly ($P < 0.05$) affected by the diet. Feed conversion ratio (FCR), protein efficiency ratio (PER), energy efficiency ratio (EER), protein retention (PR) and energy retention (ER) were significantly ($P < 0.05$) affected by both diet type and Se supplementation. Fish fed diet supplemented with 1.0 mg/kg Se

attained significantly higher FCR than the fish fed lower Se-supplemented diets. Conversely, snapper fed diets supplemented with 0 and 0.8 mg/kg Se resulted in significantly ($P > 0.05$) higher PER, EER, PR and ER than the fish fed diet supplemented with 1.0 mg/kg Se. Relative to CD diet, snapper fed BS diet had significantly higher PER, EER, PR and lower FCR. There was no significant ($P > 0.05$) interaction between diet types and Se supplementation on FI, FCR, PER, EER, PR and ER in snapper.

4.1.2 Somatic indices

The somatic indices of snapper fed test diets are presented in Table 4.1.3. There were no significant ($P > 0.05$) effects of diet types, Se supplementation and their interaction on the viscerasomatic index (VSI) and condition factor (CF) of snapper after 10-weeks of feeding. Snapper fed CD diet had significantly ($P < 0.05$) lower hepatosomatic index (HSI) than the fish fed BS diet.

Table 4.1.3 Somatic indices and Se contents of Australian snapper fed diets with Se supplementation

	VSI (%)	HSI (%)	CF (%)	CV (%)	TGC (day 1-70; 24.70 ^{0}C)
Diets					
CD0	8.95	1.20	3.76	10.58	0.51
CD0.8	9.00	1.26	3.89	10.84	0.55
CD1.0	8.69	1.17	3.81	13.33	0.41
BS0	8.72	1.36	3.81	9.79	0.52
BS0.8	8.37	1.33	3.86	10.61	0.52
BS1.0	9.14	1.28	3.73	18.65	0.38
S.E.M	0.13	0.02	0.02	0.907	0.018
Means of main effects of diet types					
CD	8.88	1.21^a	3.82	11.58	0.49
BS	8.74	1.33^b	3.80	13.02	0.47
Means of main effects of Se					
0	8.34	1.28	3.78	10.19^x	0.51^y
0.8	8.68	1.30	3.88	10.73^x	0.53^y
1.0	8.91	1.23	3.77	15.99^y	0.40^x
Two-ways ANOVA: P-values					
Diet types	0.619	0.021	0.661	0.274	0.572
Se	0.780	0.392	0.141	0.005	0.001
Diet types X Se	0.282	0.741	0.497	0.132	0.652

Values are displayed as mean of triplicate groups. Means with different alphabets (a, b or x, y) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of diet types and dietary Se supplementation, respectively. VSI Viscerasomatic index; HSI Hepatosomatic index; CF Condition factor; CV Coefficient of variances; TGC Thermal growth coefficient; S.E.M Standard error of means; Se Selenium; CD Reconstituted commercial diet; BS Basal diet.

The coefficient of variation (CV) and thermal growth coefficient (TGC) were not significantly ($P > 0.05$) affected by the diet types and the interaction between diet types and Se supplementation. Fish fed diet supplemented with 1.0 mg/kg Se showed significantly ($P < 0.05$) greater CV than the fish fed lower Se-supplemented diets. In contrast, the significantly ($P < 0.05$) lower TGC was observed in snapper fed diets supplemented 1.0 mg/kg Se.

4.1.3 Proximate composition

The crude protein, lipid and ash in the whole-body were not significantly ($P > 0.05$) affected by the diet types, Se supplementation and their interaction (Table 4.1.4). The dry matter and gross energy in the whole body were significantly ($P < 0.05$) affected by both diet types and Se supplementation. Fish fed diet supplemented with 1.0 mg/kg Se resulted in a lower dry matter and gross energy in the body than the fish fed diets supplemented with 0.8 mg/kg Se. There were no significant ($P > 0.05$) effects of diet types, Se supplementation and their interaction on the crude protein, lipid, ash, dry matter and gross energy in the muscle tissues (Table 4.1.5).

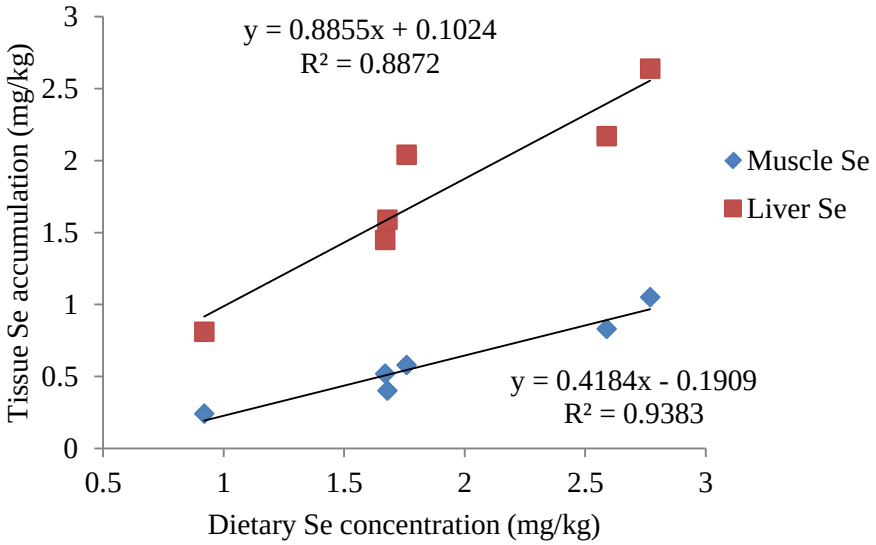


Figure 4.1.1 Linear regression between dietary Se concentration and tissue Se depositions in snapper

Dietary Se supplementation significantly ($P < 0.05$) affected the lipid content in the liver of snapper, where the fish fed diet supplemented with 1.0 mg/kg Se resulted in

significantly higher hepatic lipid concentration than the fish fed diets supplemented with 0 and 0.8 mg/kg Se. However, the liver lipid levels were not significantly ($P > 0.05$) affected by the diet types and the interaction between diet types and Se supplementation. The tissue Se depositions were significantly ($P < 0.05$) affected by both diet types and Se supplementation, but were not significantly ($P > 0.05$) influenced by their interaction (Table 4.1.5). Snapper fed diet supplemented with 1.0 mg/kg Se resulted in the highest Se concentrations in both liver and muscle tissues than the fish fed lower Se-supplemented diets. Snapper fed the BS diet attained higher tissue Se accumulations than the fish fed CD diet. Linear regression analysis showed a strong relationship between dietary Se levels and tissues Se accumulation ($y = 0.4184x – 0.1909$, $R^2 = 0.9383$ and $P < 0.05$ for muscle Se; $y = 0.8855x + 0.1024$, $R^2 = 0.8872$ and $P < 0.05$ for liver Se; Figure 4.1.1).

Table 4.1.4 Body compositions of Australian snapper fed diets with Se supplementation

	Crude protein (%)	Crude lipid (%)	Dry matter (%)	Ash (%)	Gross energy (MJ/kg)
Diets					
CD0	17.12	13.04	36.97	5.39	9.33
CD0.8	17.16	13.86	38.41	5.51	10.07
CD1.0	17.19	13.72	37.27	5.43	9.60
BS0	17.21	13.80	36.23	5.40	9.29
BS0.8	17.59	12.43	36.93	5.27	9.56
BS1.0	16.44	12.65	34.36	5.09	8.82
S.E.M	1.25	3.56	3.67	0.54	0.115
Means of main effects of diet types					
CD	17.16	13.54	37.55^{b}	5.44	9.67^{b}
BS	17.08	12.95	35.84^{a}	5.26	9.22^{a}
Means of main effects of Se					
0	17.16	13.42	36.60^{xy}	5.40	9.31^{xy}
0.8	17.38	13.15	37.67^{y}	5.39	9.81^{y}
1.0	16.81	13.17	35.81^{x}	5.26	9.21^{x}
Two-ways ANOVA: P-values					
Diet types	0.727	0.466	0.006	0.094	0.021
Se	0.157	0.950	0.036	0.494	0.027
Diet types X Se	0.128	0.489	0.251	0.380	0.222

Values are displayed as mean of triplicate groups. Means with different alphabets (a, b or x, y) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of diet types and dietary Se supplementation, respectively. S.E.M Standard error of means; Se Selenium; CD Reconstituted commercial diet; BS Basal diet.

Table 4.1.5 Muscle composition, liver lipid and tissue Se depositions in Australian snapper fed diets with Se supplementation

	Crude protein (g/kg)	Crude lipid (g/kg)	Dry matter (g/kg)	Ash (g/kg)	Gross energy (MJ/kg)	Liver lipid (g/kg)	Muscle Se (mg/kg)	Liver Se (mg/kg)
Diets								
CD0	20.39	4.18	26.15	1.52	5.92	19.57	0.24	0.81
CD0.8	19.88	4.43	25.95	1.51	5.91	19.95	0.40	1.59
CD1.0	20.30	4.45	26.27	1.68	5.86	21.53	0.58	2.04
BS0	20.35	4.17	26.64	1.64	6.05	19.58	0.52	1.45
BS0.8	19.92	4.30	25.81	1.51	5.92	20.49	0.83	2.17
BS1.0	20.33	4.47	26.46	1.61	6.04	22.48	1.05	2.64
S.E.M	1.06	0.21	0.96	0.36	0.03	2.87	0.15	0.07
Means of main effects of diet types								
CD	20.19	4.35	26.12	1.57	5.90	20.35	0.41^{a}	1.48^{a}
BS	20.20	4.31	26.3.0	1.59	6.00	20.85	0.80^{b}	2.09^{b}
Means of main effects of Se								
0	20.37	4.17	26.39	1.58	5.99	19.58^{x}	0.38^{x}	1.13^{x}
0.8	19.90	4.37	25.88	1.51	5.91	20.22^{x}	0.62^{y}	1.88^{y}
1.0	20.32	4.46	26.37	1.64	5.95	22.01^{y}	0.82^{z}	2.34^{z}
Two-ways ANOVA: P-values								
Diet types	0.976	0.918	0.303	0.851	0.092	0.105	0.000	0.000
Se	0.211	0.814	0.051	0.397	0.556	0.000	0.000	0.000
Diet types X Se	0.985	0.986	0.344	0.555	0.472	0.433	0.322	0.981

Values are displayed as mean of triplicate groups. Means with different alphabets (a, b or x, y, z) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of diet types and dietary Se supplementation, respectively. S.E.M Standard error of means; Se Selenium; CD Reconstituted commercial diet; BS Basal diet.

4.1.4 Histological evaluation

Snapper fed reconstituted commercial diet with and without Se supplementation did not show any histopathological lesions on the livers (Figure 4.1.2), whereas the increases in lipid droplet accumulation were observed in livers of snapper fed the basal diet supplemented with 0.8 and 1.0 mg/kg Se (Figure 4.1.3 and Figure 4.1.4).

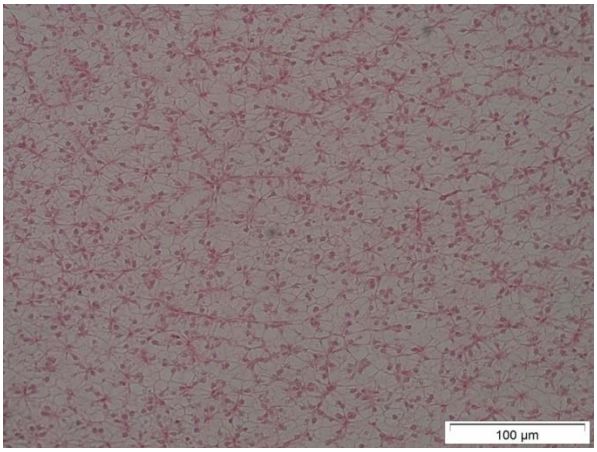


Figure 4.1.2 Liver section of snapper fed reconstituted commercial diet without Se adding show normal hepatocytes.

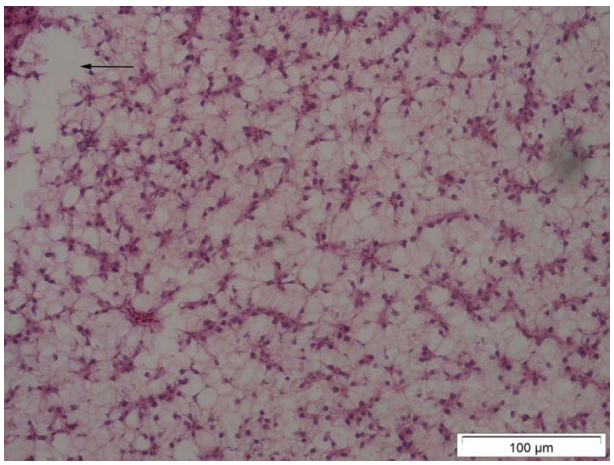


Figure 4.1.3 The increased hepatic lipid accumulation in snapper fed the basal diet with 0.8 mg/kg Se supplementation (arrow).

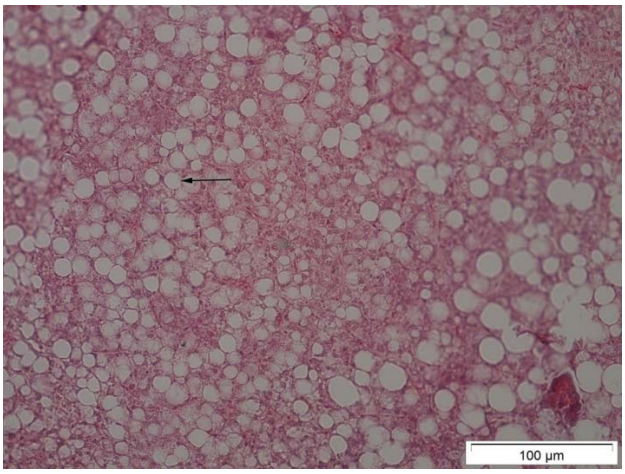


Figure 4.1.4 Liver of snapper fed the basal diet with 1.0 mg/kg Se supplementation show vacuoles degeneration (VD) with hepatocyte reduction (arrow).

4.2 EXPERIMENT 2: NUTRITIONAL ROLES OF SELENIUM SUPPLEMENTATION ON GROWTH AND PHYSIOLOGICAL PERFORMANCES OF COBIA

4.2.1 Growth performances and feed utilization

Dietary Se concentration significantly ($P < 0.05$) affected the growth and feed utilisation of cobia after 8 weeks of feeding (Table 4.2.1 and Table 4.2.2).

Table 4.2.1 Growth performances of cobia fed diets containing various inclusion levels of Se for 8 weeks

Dietary Se (mg/kg)	IBW (g/fish)	FBW (g/fish)	SGR (%/day)	WG	Survival
1.15	13.61 ± 0.05	84.26 ± 0.54^{a}	3.26 ± 0.01^{a}	70.66 ± 0.53^{a}	97.22 ± 2.78
1.52	13.72 ± 0.02	86.92 ± 0.48^{ab}	3.30 ± 0.02^{a}	73.20 ± 0.49^{ab}	100
1.93	13.61 ± 0.05	94.41 ± 0.69^{c}	3.46 ± 0.02^{c}	80.80 ± 0.73^{c}	100
2.29	13.71 ± 0.05	93.88 ± 0.73^{c}	3.44 ± 0.02^{c}	80.17 ± 0.76^{c}	100
2.71	13.65 ± 0.05	91.04 ± 1.41^{bc}	3.39 ± 0.03^{bc}	77.39 ± 1.41^{bc}	100
3.14	13.61 ± 0.02	88.87 ± 2.74^{abc}	3.35 ± 0.05^{abc}	75.27 ± 2.69^{abc}	94.44 ± 2.78

Data represent mean ± SE. Values in the same column with different superscripts are significantly different ($P < 0.05$). IBW: Initial body weight, FBW: Final body weight, SGR: Specific growth rate, WG: Weight gain.

Cobia fed dietary Se of 1.93, 2.29 and 2.71 mg/kg Se showed significantly higher FBW, SGR, WG and FI than the fish fed the control diet. Fish fed the highest dietary Se level started showing the reduction in the FBW, SGR and WG. Dietary Se supplementation did not affect the FCR, PR and ER in cobia. High survival rates obtained in all dietary treatments, ranging from 91.67 to 100 %, and was not significantly affected by any

dietary Se levels. The optimum dietary Se required for maximal SGR in juvenile cobia was 2.32 mg/kg, based on the second-degree polynomial regression analysis (Figure 4.2.1).

Table 4.2.2 Feed utilization of cobia fed diets containing various inclusion levels of Se for 8 weeks

Dietary Se (mg/kg)	FI (g/fish)	FCR	PER	EER	PR (%)	ER (%)
1.15	88.57 ± 2.97^{a}	1.30 ± 0.01	1.51 ± 0.05	3.71 ± 0.13	25.85 ± 0.97	24.69 ± 0.70
1.52	94.67 ± 1.17^{ab}	1.29 ± 0.01	1.47 ± 0.01	3.59 ± 0.02	25.33 ± 0.16	23.96 ± 0.17
1.93	104.24 ± 1.31^{b}	1.29 ± 0.01	1.47 ± 0.01	3.60 ± 0.02	24.99 ± 0.18	23.80 ± 0.45
2.29	104.49 ± 1.15^{b}	1.30 ± 0.01	1.45 ± 0.01	3.56 ± 0.01	24.41 ± 0.21	23.09 ± 0.15
2.71	103.78 ± 2.48^{b}	1.31 ± 0.01	1.41 ± 0.04	3.47 ± 0.10	24.28 ± 0.92	22.97 ± 0.66
3.14	96.21 ± 1.71^{ab}	1.31 ± 0.01	1.48 ± 0.02	3.64 ± 0.04	24.72 ± 0.18	23.65 ± 0.45

Data represent mean ± SE. Values in the same column with different superscripts are significantly different ($P < 0.05$). FI: feed intake, FCR: feed conversion ratio, PR: Protein retention and ER: Energy retention.

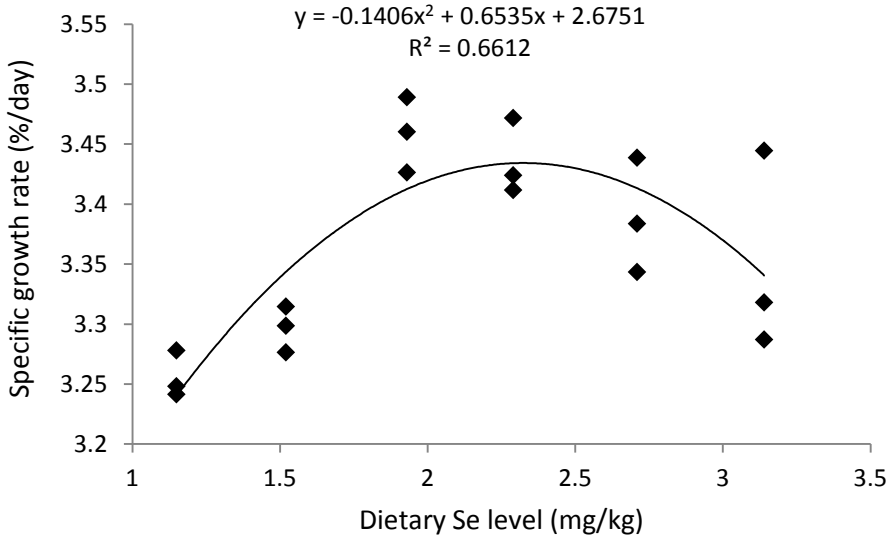


Figure 4.2.1 The quadratic regression between dietary Se level and specific growth rate of juvenile cobia after 8 weeks

4.2.2 Body and muscle composition

The supplementation of organic Se had no significant ($P > 0.05$) effects on the proximate compositions in the whole-body and muscle tissues (Table 4.2.3). The increased hepatic lipid concentration was found in the fish fed the highest dietary level. The tissue Se depositions significantly ($P < 0.05$) increased corresponding with increasing dietary Se level (Table 4.2.4). The regression analysis showed positive linear relationship between

tissue Se levels and dietary Se concentrations ($y = 0.5399x + 0.5569$, $R^2 = 0.967$ and $P < 0.001$ for the liver Se and $y = 0.451x – 0.1359$, $R^2 = 0.9599$ and $P < 0.001$ for the muscle; Figure 4.2.2).

Table 4.2.3 Whole-body and muscle compositions of cobia fed test diets

Dietary Se (mg/kg)	Crude protein (%)	Crude lipid (%)	Dry matter (%)	Ash (%)	Gross energy MJ/kg
Whole body					
1.15	16.78 ± 0.12	6.34 ± 0.06	28.68 ± 0.20	3.22 ± 0.06	6.39 ± 0.03
1.52	16.98 ± 0.01	6.36 ± 0.05	29.13 ± 0.46	3.17 ± 0.02	6.41 ± 0.03
1.93	16.77 ± 0.12	6.38 ± 0.02	29.02 ± 0.44	3.15 ± 0.09	6.38 ± 0.08
2.29	16.57 ± 0.09	6.47 ± 0.02	30.06 ± 0.16	3.13 ± 0.07	6.27 ± 0.03
2.71	16.88 ± 0.13	6.43 ± 0.07	29.84 ± 0.27	3.31 ± 0.06	6.38 ± 0.04
3.14	16.46 ± 0.07	6.53 ± 0.07	28.69 ± 0.19	3.05 ± 0.17	6.26 ± 0.04
Muscle composition					
1.15	18.41 ± 0.18	2.26 ± 0.01	24.29 ± 0.25	1.18 ± 0.06	5.38 ± 0.06
1.52	18.74 ± 0.25	2.26 ± 0.06	23.73 ± 0.26	1.17 ± 0.07	5.51 ± 0.07
1.93	18.45 ± 0.35	2.25 ± 0.08	23.22 ± 0.54	1.23 ± 0.05	5.44 ± 0.13
2.29	18.32 ± 0.22	2.34 ± 0.02	23.25 ± 0.52	1.21 ± 0.06	5.33 ± 0.20
2.71	18.30 ± 0.29	2.30 ± 0.06	23.01 ± 0.33	1.22 ± 0.03	5.25 ± 0.05
3.14	18.15 ± 0.25	2.25 ± 0.03	23.05 ± 0.62	1.17 ± 0.09	5.10 ± 0.08

Data represent mean ± SE. Values in the same column with different superscripts are significantly different ($P < 0.05$).

Table 4.2.4 Liver lipid and Se accumulation (dry weight) in the tissues

Dietary Se (mg/kg)	Liver lipid (%)	Liver Se (mg/kg)	Muscle Se (mg/kg)	GPx (U/g Hb)
1.15	24.03 ± 0.29^{a}	1.22 ± 0.02^{a}	0.44 ± 0.02^{a}	54.57 ± 1.79^{a}
1.52	24.20 ± 0.38^{ab}	1.37 ± 0.01^{b}	0.54 ± 0.01^{a}	67.65 ± 1.69^{b}
1.93	24.27 ± 0.50^{ab}	1.51 ± 0.02^{c}	0.66 ± 0.02^{b}	68.88 ± 2.58^{b}
2.29	24.43 ± 0.12^{ab}	1.79 ± 0.02^{d}	0.85 ± 0.04^{c}	75.92 ± 1.37^{bc}
2.71	25.43 ± 0.52^{ab}	2.12 ± 0.03^{e}	1.16 ± 0.03^{d}	82.27 ± 2.36^{cd}
3.14	25.93 ± 0.24^{b}	2.21 ± 0.08^{e}	1.28 ± 0.02^{e}	88.58 ± 0.88^{d}

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets within a column are significantly different ($P < 0.05$).

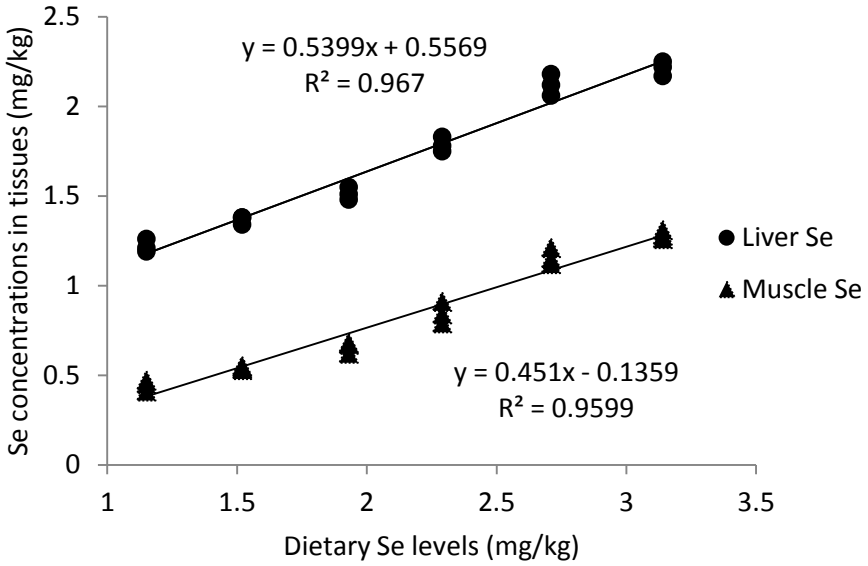


Figure 4.2.2 Relationship between dietary Se concentration and tissue Se accumulations of juvenile cobia after 8 weeks.

4.2.3 Haematological and glutathione peroxidase responses

Dietary Se supplementation significantly affected the physiological performances of cobia (Table 4.2.5). The fish fed 3.14 mg/kg Se diet resulted in the reduction ($P > 0.05$) of Ht. Increasing dietary Se from 1.52 to 2.71 mg/kg Se resulted in significantly ($P < 0.05$) higher RBC levels than the fish fed 3.14 mg/kg Se diet. The Hb significantly increased and peaked in cobia fed 1.52 mg/kg Se diet, but significantly reduced in fish fed the highest dietary level of Se. The dietary Se supplementation had no effects on values of the mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

Table 4.2.5 Haematological parameters of cobia fed test diets

Dietary Se (mg/kg)	Ht (%)	RBC ($x10^{12}$/L)	Hb (g/dl)	MCV (fl)	MHCV (g/dl)
1.15	39.23 ± 0.24^{ab}	3.78 ± 0.02^{ab}	6.33 ± 0.09^{a}	103.89 ± 1.01	16.14 ± 0.13
1.52	40.30 ± 0.59^{ab}	3.92 ± 0.03^{bc}	7.13 ± 0.18^{c}	102.92 ± 2.09	17.71 ± 0.47
1.93	40.97 ± 0.39^{b}	4.01 ± 0.02^{c}	7.00 ± 0.15^{c}	102.26 ± 1.38	17.09 ± 0.48
2.29	39.67 ± 0.56^{ab}	3.92 ± 0.04^{bc}	6.97 ± 0.07^{bc}	101.27 ± 0.38	17.57 ± 0.42
2.71	39.10 ± 0.26^{ab}	3.93 ± 0.02^{c}	6.70 ± 0.10^{abc}	99.47 ± 0.78	17.14 ± 0.35
3.14	38.50 ± 0.40^{a}	3.73 ± 0.04^{a}	6.37 ± 0.17^{ab}	103.15 ± 1.53	16.54 ± 0.46

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets within a column are significantly different ($P < 0.05$). Ht: Haematocrit, RBC: Red blood cell, Hb: Haemoglobin, MCV: Mean corpuscular volume, MCHC: Mean corpuscular haemoglobin concentration.

The lowest GPx was observed in cobia fed the control diet. The GPx activity significantly ($P < 0.05$) increased and gained a plateau in the fish fed dietary Se levels of between 1.53 and 2.29 mg/kg, and then increased in cobia fed 2.71 mg/kg and above this level (Table 4.2.4).

4.2.4 Somatic indices

The dietary Se supplementation had no significant ($P > 0.05$) effects in the VSI, CF and CV at the end of the feeding. The HSI was significantly ($P < 0.05$) lower in cobia fed the highest dietary level of Se than the fish fed 1.93 mg/kg Se diet. The cobia fed the dietary Se levels of between 1.93 and 2.71 mg/kg resulted in significantly higher TGC than the fish fed the control diet (Table 4.2.6).

Table 4.2.6 Somatic indices, condition factor, coefficient of variances and thermal growth coefficient of cobia fed test diets

Dietary Se (mg/kg)	VSI (%)	HSI (%)	CF (%)	CV (%)	TGC (%)
1.15	11.89 ± 0.20	2.12 ± 0.03^{ab}	0.71 ± 0.01^{b}	8.93 ± 0.95	1.26 ± 0.01^{a}
1.52	11.86 ± 0.29	2.09 ± 0.07^{ab}	0.71 ± 0.01^{b}	5.66 ± 1.39	1.29 ± 0.01^{ab}
1.93	11.44 ± 0.04	2.14 ± 0.09^{b}	0.72 ± 0.01^{b}	6.95 ± 1.15	1.37 ± 0.01^{c}
2.29	11.61 ± 0.14	2.13 ± 0.02^{ab}	0.68 ± 0.01^{ab}	7.85 ± 0.47	1.36 ± 0.01^{c}
2.71	11.22 ± 0.05	1.91 ± 0.02^{ab}	0.66 ± 0.01^{a}	7.93 ± 0.36	1.34 ± 0.02^{bc}
3.14	10.00 ± 0.15	1.90 ± 0.01^{a}	0.64 ± 0.01^{a}	8.47 ± 0.24	1.31 ± 0.03^{abc}

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets within a column are significantly different ($P < 0.05$). VSI: Viscerasomatic index, HSI: Hepatosomatic index, CF: Condition factor, CV: Coefficient of variances, TGC: Thermal growth coefficient.

4.2.5 Histological examination

The cobia fed various dietary Se inclusion levels did not show any histological alteration in the intestinal tissues. Fish fed the control diet containing 1.15 mg/kg Se had normal histological appearance in the hepatic tissue (Figure 4.2.3), whereas, the cell necrosis and dilation of bile duct were observed in the liver of cobia fed the highest Se level (Figure 4.2.4 and Figure 4.2.5).

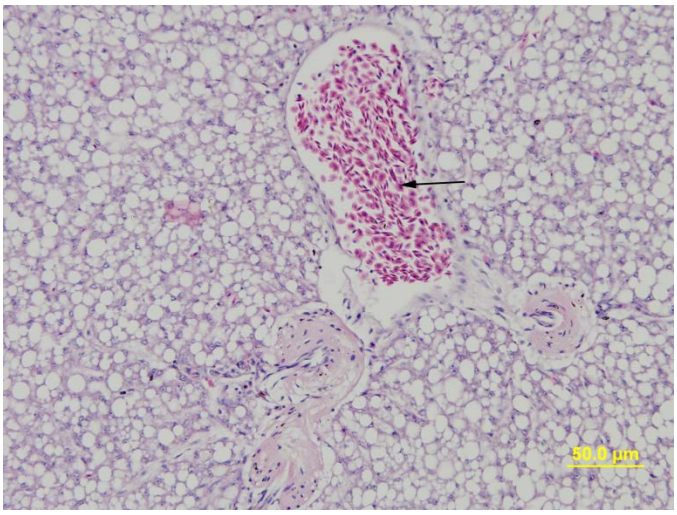


Figure 4.2.3 Liver section of cobia fed a commercial diet containing 1.15 mg/kg Se showed normal hepatocytes (arrow). Haematoxylin and eosin.

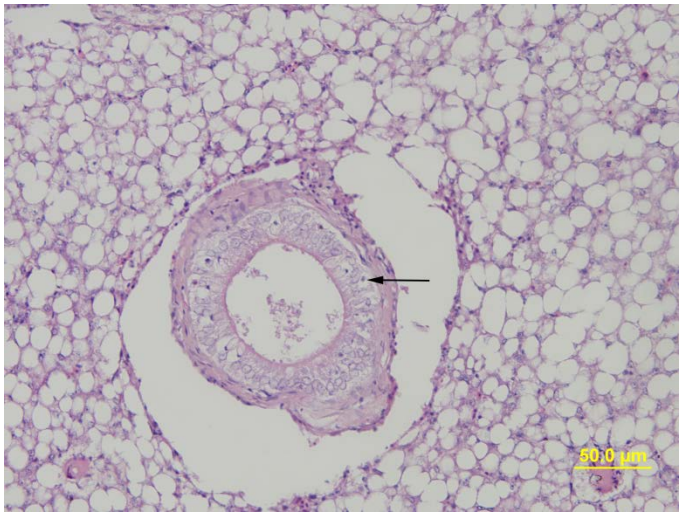


Figure 4.2.4 Sections of liver tissues of cobia fed the highest Se level started showing the dilation of bile duct (arrow). Haematoxylin and eosin.

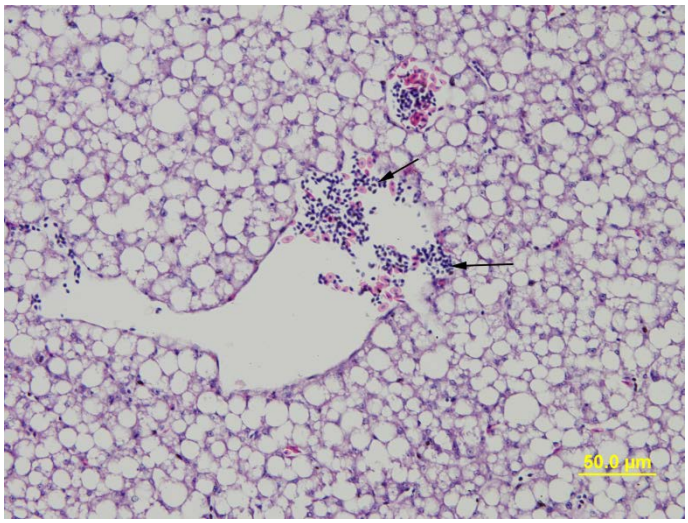


Figure 4.2.5 Sections of liver tissues of cobia fed the highest Se level showed the necrotic hepatocytes (arrows). Haematoxylin and eosin.

4.3 EXPERIMENT 3 & 4: NUTRTIONAL AND PHYSIOLOGICAL RESPONSES OF SNAPPER AND COBIA FED DIETARY LUPIN KERNEL MEAL LEVELS

4.3.1 Growth and feed utilisation

Growth and feed utilisation efficiency of snapper and cobia fed various dietary LKM levels are presented in Table 4.3.1 and Table 4.3.2. The FBW, SGR and WG of snapper were significantly ($P < 0.05$) reduced with an increase in the inclusion levels of LKM in the test diets. However, there were no significant ($P > 0.05$) differences in the growth performance of snapper fed the LKM315 and LKM420 diets. No significant difference was observed in the final weight and WG of cobia fed diets containing 0 and 105 g/kg LKM, but these factors significantly decreased in the fish fed diets with more than 105 g/kg LKM compared to the fish fed the control diet. The SGR of the cobia fed LKM0, LKM105 and LKM210 diets were significantly higher than the fish fed diets with more than 210 g/kg LKM.

Table 4.3.1 Growth performances of snapper and cobia fed test diets.

	Diet group				
	LKM0	LKM105	LKM210	LKM315	LKM420
FBW_S	${}_A75.88 \pm 1.67^d$	${}_A64.36 \pm 0.98^c$	${}_A56.94 \pm 1.79^b$	${}_A44.96 \pm 1.10^a$	${}_A39.98 \pm 0.30^a$
FBW_C	${}_B114.13 \pm 2.41^d$	${}_B111.60 \pm 1.24^d$	${}_B101.20 \pm 1.46^c$	${}_B66.99 \pm 2.82^b$	${}_B57.07 \pm 2.25^a$
SGR_S	${}_A1.80 \pm 0.03^d$	${}_A1.60 \pm 0.05^c$	${}_A1.37 \pm 0.07^b$	${}_A1.02 \pm 0.03^a$	${}_A0.88 \pm 0.02^a$
SGR_C	${}_B3.29 \pm 0.04^c$	${}_B3.26 \pm 0.02^c$	${}_B3.08 \pm 0.03^c$	${}_B2.34 \pm 0.07^b$	${}_B2.05 \pm 0.06^a$
WG_S	${}_A0.78 \pm 0.02^d$	${}_A0.62 \pm 0.02^c$	${}_A0.50 \pm 0.02^b$	${}_A0.33 \pm 0.01^a$	${}_A0.26 \pm 0.01^a$
WG_C	${}_B1.72 \pm 0.04^d$	${}_B1.67 \pm 0.02^d$	${}_B1.48 \pm 0.03^c$	${}_B0.87 \pm 0.05^b$	${}_B0.70 \pm 0.04^a$
$Survival_S$	100	100	100	100	100
$Survival_C$	97.22 ± 2.78	100	94.44 ± 2.78	91.67 ± 4.81	88.89 ± 5.55

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a row are significantly different ($P < 0.05$). Mean with different upper case alphabets (A, B) within a similar parameter show the significant difference between snapper and cobia at each inclusion level of lupin meal. Final body weight (FBW), Specific growth rate (SGR), weight gain (WG).

The high survival rates were obtained in both snapper (100 %) and cobia (88.89 – 100 %), and no significant differences were recorded in either of the species fed different diets at the end of the feeding period. The correlation between the SGR and dietary LKM in snapper ($R^2 = 0.97$) was significantly stronger than in cobia ($R^2 = 0.87$) (Figure 4.3.1). The SGR and WG in cobia were significantly greater than in the snapper at each

inclusion level of lupin meal, whereas the survival rate did not differ between these species.

Table 4.3.2 Feed utilisation of snapper and cobia fed test diets.

	Diet group				
	LKM0	LKM105	LKM210	LKM315	LKM420
FI_S	$_A56.05 \pm 2.34^c$	$_A45.07 \pm 2.81^b$	$_A42.57 \pm 0.90^b$	$_A33.98 \pm 0.68^a$	$_A30.98 \pm 0.60^a$
FI_C	$_B137.95 \pm 2.90^b$	$_B135.52 \pm 1.29^b$	$_B128.05 \pm 3.27^b$	$_B81.01 \pm 4.77^a$	$_B68.32 \pm 4.05^a$
VFI_S	$_A1.64 \pm 0.05$	$_A1.51 \pm 0.08$	$_A1.55 \pm 0.01$	$_A1.45 \pm 0.01$	$_A1.44 \pm 0.05$
VFI_C	$_B3.73 \pm 0.06^b$	$_B3.73 \pm 0.01^b$	$_B3.83 \pm 0.06^b$	$_B3.39 \pm 0.09^a$	$_B3.24 \pm 0.09^a$
FCR_S	$_A1.03 \pm 0.02^a$	$_A1.04 \pm 0.03^a$	$_A1.21 \pm 0.04^b$	$_A1.48 \pm 0.03^c$	$_A1.69 \pm 0.01^d$
FCR_C	$_B1.44 \pm 0.03^a$	$_B1.45 \pm 0.01^a$	1.54 ± 0.02^b	$_B1.66 \pm 0.01^c$	$_B1.75 \pm 0.01^d$
PER_S	$_B2.09 \pm 0.03^d$	$_B2.07 \pm 0.06^d$	$_B1.76 \pm 0.05^c$	$_B1.46 \pm 0.03^b$	$_B1.27 \pm 0.01^a$
PER_C	$_A1.50 \pm 0.03^d$	$_A1.48 \pm 0.01^d$	$_A1.39 \pm 0.02^c$	$_A1.31 \pm 0.01^b$	$_A1.22 \pm 0.01^a$
PR_S	$_B37.15 \pm 1.30^c$	$_B36.80 \pm 0.34^c$	$_B31.28 \pm 0.45^b$	$_B25.65 \pm 0.87^a$	$_B22.54 \pm 0.52^a$
PR_C	$_A23.39 \pm 0.02^d$	$_A24.07 \pm 0.15^d$	$_A22.23 \pm 0.27^c$	$_A19.18 \pm 0.17^b$	$_A17.99 \pm 0.18^a$
ER_S	$_B44.64 \pm 1.34^c$	$_B38.95 \pm 1.09^b$	$_B35.88 \pm 1.34^b$	$_B26.01 \pm 1.43^a$	$_B23.89 \pm 1.10^a$
ER_C	$_A22.50 \pm 0.82^c$	$_A21.38 \pm 0.11^{bc}$	$_A19.45 \pm 0.23^a$	$_A19.74 \pm 0.23^{ab}$	$_A17.87 \pm 0.23^a$

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a row are significantly different ($P < 0.05$). Mean with different upper case alphabets (A, B) within a similar parameter show the significant difference between snapper and cobia at each inclusion level of lupin meal. Feed intake (FI), Feed conversion ratio (FCR), VFI: Voluntary feed intake, Protein efficiency ratio (PER), Protein retention (PR), Energy retention (ER).

The highest FI was found in the snapper fed the LKM0 diet and was significantly ($P < 0.05$) higher than the fish fed other diets, but no significant differences were seen on the voluntary feed intake (VFI) of snapper at the end of feeding period (Table 4.3.2). There was no significant effect on the FCR of the snapper fed the diets containing 0 and 105 g/kg LKM. The FCR of snapper significantly increased as the dietary LKM exceeded 105 g/kg. The PER values of the snapper fed the LKM0 and LKM105 diets were significantly higher than those of the snapper fed the LKM210, LKM315 and LKM420 diets. The ER and PR values were significantly lower in the snapper fed diets containing LKM than in the fish fed the control diet. In cobia, the FI and VFI significantly decreased when fed diets including more than 210 g/kg LKM. The FCR of the cobia fed the LKM105 diet was comparable to that of the fish in the control group and significantly lower than that of the fish fed higher inclusion levels of LKM. The negative responses on the PER, PR and ER were recorded in the cobia fed diets containing more than 105 g/kg LKM. In terms of species differences, the FI, VFI and FCR in the cobia

were significantly higher than in the snapper, whereas PER, PR and ER were lower in the cobia than in the snapper at each inclusion level of lupin meal. There were no significant differences in the correlation coefficient between the FCR or PER and dietary LKM between the two species (Figure 4.3.1).

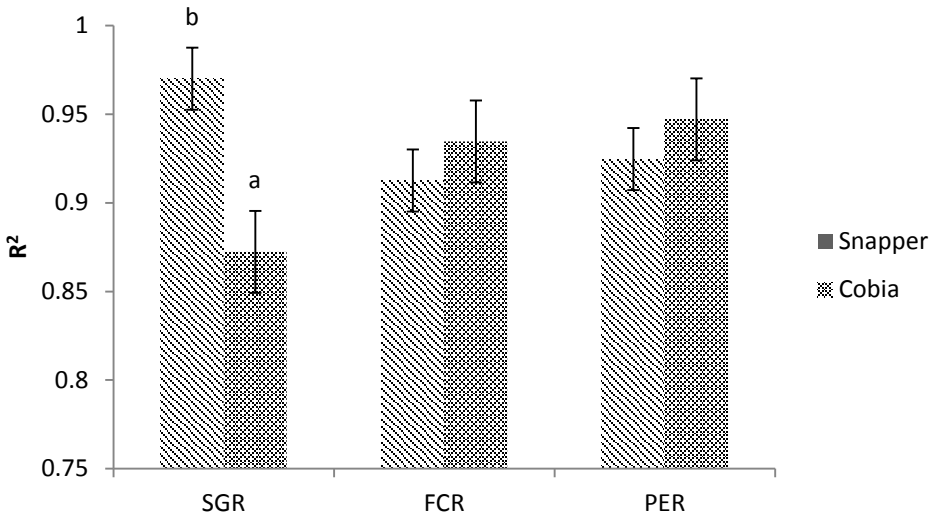


Figure 4.3.1 The relative R^2 values of the SGR, FCR and PER of snapper and cobia fed dietary lupin kernel meal inclusions. Bars with same letters are not significantly different.

4.3.2 Digestibility

The ADCs of protein and energy in the snapper fed diets with lupin meal supplements were significantly ($P < 0.05$) lower than in those fed the control diet, while the ADCs of protein and energy were significantly reduced in the cobia fed diets containing more than 105 g/kg lupin meal inclusions. The dietary lupin meal supplements had no significant effect on the ADC of dry matter of the snapper, but did affect the cobia (Table 4.3.3).

The ADC of the ingredients was not different when the protein digestibility of the lupin kernel meal in either snapper or cobia was assessed (Table 4.3.3). Similar results were observed for the ADC of energy in the cobia, while the energy digestibility of the lupin meal in the snapper fed the LKM105 diet were significantly higher than for those fed the LKM315 and LKM420 diets. The dry matter digestibility of lupin in the cobia was lower in LKM315 and LKM420 than LKM105, but there was no change in the ADC of dry matter in the snapper (Table 4.3.3). In term of species differences, the protein and dry matter digestibility of lupin meal was not different between snapper and cobia fed the LKM105 and LKM210 diets, whereas the ADC of protein and the dry matter of lupin meal were significantly higher in snapper than in cobia for the LKM315 and LKM420

groups. The ADC of energy of lupin meal for the snapper fed the LKM105 and LKM210 diets were significantly higher than for the cobia fed the same inclusion level of lupin, whereas the ADC of energy of lupin meal did not show any differences between snapper and cobia when the fish were fed the LKM315 and LKM420 diets (Table 4.3.3).

Table 4.3.3 Apparent digestibility coefficients (ADCs) of diets and ingredient for snapper and cobia.

Nutrients	Diet group				
	LKM0	LKM105	LKM210	LKM315	LKM420
Diet ADC (%)					
$Protein_S$	$_{B}93.33 \pm 0.17^{c}$	$_{B}91.14 \pm 0.37^{b}$	$_{B}90.91 \pm 0.48^{b}$	$_{B}88.23 \pm 0.27^{a}$	$_{B}88.10 \pm 0.21^{a}$
$Protein_C$	$_{A}89.82 \pm 0.66^{c}$	$_{A}87.73 \pm 0.44^{bc}$	$_{A}85.99 \pm 0.82^{b}$	$_{A}82.76 \pm 0.53^{a}$	$_{A}81.57 \pm 0.18^{a}$
$Energy_S$	$_{B}82.08 \pm 0.22^{c}$	$_{B}78.42 \pm 0.13^{b}$	$_{B}76.02 \pm 0.18^{b}$	$_{B}70.86 \pm 0.22^{a}$	$_{B}69.23 \pm 1.27^{a}$
$Energy_C$	$_{A}74.61 \pm 1.14^{d}$	71.48 ± 0.62^{cd}	$_{A}68.36 \pm 0.42^{bc}$	$_{A}65.49 \pm 1.21^{ab}$	$_{A}62.43 \pm 0.67^{a}$
DM_S	$_{B}69.49 \pm 0.69$	$_{B}68.95 \pm 0.76$	$_{B}68.97 \pm 1.56$	$_{B}67.41 \pm 1.31$	$_{B}67.33 \pm 0.84$
DM_C	$_{A}66.05 \pm 0.62^{c}$	$_{A}65.19 \pm 0.36^{bc}$	$_{A}63.98 \pm 0.55^{b}$	$_{A}61.08 \pm 0.20^{a}$	$_{A}60.06 \pm 0.45^{a}$
Ingredient ADC (%)					
$Protein_S$	-	88.38 ± 4.20	97.50 ± 1.16	$_{B}89.82 \pm 1.01$	$_{B}97.36 \pm 0.61$
$Protein_C$	-	85.15 ± 5.02	88.51 ± 4.72	$_{A}78.31 \pm 1.98$	$_{A}84.53 \pm 0.52$
$Energy_S$	-	$_{B}76.34 \pm 1.32^{c}$	$_{B}74.91 \pm 0.89^{bc}$	61.97 ± 0.75^{a}	66.43 ± 3.32^{ab}
$Energy_C$	-	$_{A}68.38 \pm 1.07$	$_{A}63.02 \pm 2.13$	59.20 ± 4.09	58.59 ± 1.74
DM_S	-	68.19 ± 7.52	64.27 ± 7.61	$_{B}61.34 \pm 4.27$	$_{B}61.54 \pm 2.02$
DM_C	-	61.34 ± 3.59^{b}	53.38 ± 1.98^{ab}	$_{A}49.57 \pm 0.34^{a}$	$_{A}49.06 \pm 0.45^{a}$

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets (a, b, c, d) within a row are significantly different ($P < 0.05$). Mean with different upper case alphabets (A, B) within a similar parameter show the significant difference between snapper and cobia at each inclusion level of lupin meal. DM, Dry matter.

4.3.3 Proximate compositions

Proximate compositions in the whole body and muscle tissues of snapper and cobia are displayed in Table 4.3.4. The lipid concentrations were significantly ($P < 0.05$) lower in the whole body and muscle tissues of cobia fed diets containing more than 210 g/kg LKM compared to the fish fed the control diet, while no significant differences were observed in the lipid contents of these tissues in snapper. The liver lipid levels in the snapper fed the LKM315 and LKM420 diets were significantly higher than in the fish fed the LKM0 and LKM210 diets, whereas the LKM315 and LKM420 diets resulted in significantly lower levels than in the fish fed LKM105 the.

Table 4.3.4 Proximate composition in the whole body, muscle and liver (% or MJ/kg live-weight) of snapper and cobia fed test diets

	Diet group				
	LKM0	LKM105	LKM210	LKM315	LKM420
Whole body composition					
$Protein_S$	$_B17.42 \pm 0.27$	$_B17.39 \pm 0.32$	$_B17.28 \pm 0.19$	$_B17.04 \pm 0.12$	$_B17.09 \pm 0.20$
$Protein_C$	$_A15.51 \pm 0.26^{b}$	$_A16.02 \pm 0.03^{b}$	$_A15.82 \pm 0.06^{b}$	$_A14.74 \pm 0.16^{a}$	$_A14.74 \pm 0.06^{a}$
$Lipid_S$	$_A6.73 \pm 0.15$	$_A6.65 \pm 0.06$	$_A6.74 \pm 0.10$	6.30 ± 0.15	6.23 ± 0.18
$Lipid_C$	$_B7.64 \pm 0.11^{b}$	$_B7.69 \pm 0.20^{b}$	$_B7.40 \pm 0.03^{b}$	6.23 ± 0.08^{a}	6.11 ± 0.01^{a}
Ash_S	$_B5.03 \pm 0.12^{b}$	$_B5.07 \pm 0.03^{b}$	4.80 ± 0.04^{ab}	$_B4.79 \pm 0.07^{ab}$	$_B4.49 \pm 0.16^{a}$
Ash_C	$_A3.89 \pm 0.03^{a}$	$_A4.46 \pm 0.10^{b}$	4.73 ± 0.04^{b}	$_A3.79 \pm 0.03^{a}$	$_A3.66 \pm 0.06^{a}$
DM_S	$_B34.57 \pm 0.52^{b}$	$_B33.31 \pm 0.70^{ab}$	$_B33.85 \pm 0.29^{ab}$	$_B32.01 \pm 0.43^{a}$	$_B32.23 \pm 0.38^{a}$
DM_C	$_A29.00 \pm 0.26$	$_A29.22 \pm 0.69$	$_A29.34 \pm 0.19$	$_A29.20 \pm 0.51$	$_A28.74 \pm 0.34$
$Energy_S$	$_B9.10 \pm 0.15$	$_B8.46 \pm 0.31$	$_B8.91 \pm 0.14$	$_B8.19 \pm 0.22$	$_B8.42 \pm 0.13$
$Energy_C$	$_A6.46 \pm 0.11$	$_A6.41 \pm 0.01$	$_A6.30 \pm 0.05$	$_A6.62 \pm 0.10$	$_A6.39 \pm 0.02$
Muscle composition					
$Protein_S$	$_B21.57 \pm 0.17^{c}$	$_B21.33 \pm 0.08^{bc}$	$_B20.95 \pm 0.03^{ab}$	$_B20.56 \pm 0.20^{a}$	$_B20.58 \pm 0.07^{a}$
$Protein_C$	$_A18.02 \pm 0.28^{bc}$	$_A18.75 \pm 0.09^{c}$	$_A17.60 \pm 0.12^{ab}$	$_A16.69 \pm 0.33^{a}$	$_A16.94 \pm 0.10^{a}$
$Lipid_S$	$_A2.16 \pm 0.03$	$_A2.19 \pm 0.07$	$_A2.14 \pm 0.02$	2.23 ± 0.05	2.34 ± 0.05
$Lipid_C$	$_B2.42 \pm 0.07^{b}$	$_B2.53 \pm 0.01^{b}$	$_B2.43 \pm 0.01^{b}$	2.14 ± 0.01^{a}	2.18 ± 0.01^{a}
Ash_S	$_B1.54 \pm 0.03$	$_B1.63 \pm 0.01$	$_B1.62 \pm 0.04$	$_B1.68 \pm 0.03$	$_B1.69 \pm 0.04$
Ash_C	$_A1.12 \pm 0.04$	$_A1.04 \pm 0.01$	$_A1.29 \pm 0.02$	$_A1.05 \pm 0.09$	$_A1.23 \pm 0.12$
DM_S	25.66 ± 0.10^{b}	24.76 ± 0.44^{ab}	$_B24.73 \pm 0.07^{ab}$	$_B23.90 \pm 0.15^{a}$	$_B24.82 \pm 0.18^{ab}$
DM	23.32 ± 0.95	23.61 ± 0.35	$_A22.94 \pm 0.34$	$_A20.99 \pm 1.32$	$_A22.64 \pm 0.46$
$Energy_S$	$_B6.12 \pm 0.05$	$_B5.82 \pm 0.13$	$_B5.84 \pm 0.08$	$_B5.66 \pm 0.07$	$_B5.87 \pm 0.17$
$Energy_C$	$_A5.16 \pm 0.02^{b}$	$_A5.26 \pm 0.03^{b}$	$_A5.14 \pm 0.01^{b}$	$_A4.74 \pm 0.02^{a}$	$_A4.91 \pm 0.8^{a}$
Liver lipid levels					
$Lipid_S$	$_A16.42 \pm 0.10^{a}$	$_A16.59 \pm 0.25^{ab}$	$_A15.47 \pm 0.36^{a}$	$_A17.77 \pm 0.28^{bc}$	$_A18.59 \pm 0.26^{c}$
$Lipid_C$	$_B24.64 \pm 0.29^{ab}$	$_B25.79 \pm 0.31^{b}$	$_B24.78 \pm 0.39^{ab}$	$_B22.94 \pm 0.64^{a}$	$_B22.56 \pm 0.83^{a}$

Data represent mean ± SE. Means with different lower case alphabets (a, b, c) within a row are significantly different ($P < 0.05$). Mean with different upper case alphabets (A, B) within a similar parameter show the significant difference between snapper and cobia at each inclusion level of lupin meal ($P < 0.05$). DM, Dry matter.

The dietary lupin meal had no significant effect on the whole body protein of snapper, whereas the whole body protein contents of the cobia fed the LKM315 and LKM420 diets were significantly lower than those of the fish in other groups. The snapper fed diets containing more than 105 g/kg LKM showed reduced muscle protein, whereas this

effect was not reached until the inclusion level was 210 g/kg LKM for cobia. The dietary LKM resulted in no significant changes in the muscle ash in either of the snapper and cobia. The snapper fed diets with the highest level of LKM showed a significant decrease of whole-body ash compared to the fish fed the LKM0 and LKM105 diets. The dietary LKM had no significant effect on the gross energy of the whole body in both snapper and cobia. Similar data were also recorded for the gross energy of the muscle in snapper, while the muscle energy contents were significantly reduced in cobia fed the LKM315 and LKM420 diets.

4.3.4 Somatic indices and haematological parameters

The CV of the snapper showed high variability, ranging from 11.94 to 19.78 %, whereas there was no change ($P > 0.05$) in the CV in the cobia. The HSI of the cobia fed the LKM420 diet was significantly ($P < 0.05$) higher than the other groups, whereas the HSI of the snapper fluctuated with the increasing inclusion level of LKM. The CV values of the snapper were always significantly higher in the cobia for each level of lupin meal (Table 4.3.5).

Table 4.3.5 Somatic indices and haematological responses of snapper and cobia fed test diets

	Diet group				
	LKM0	LKM105	LKM210	LKM315	LKM420
CV_S	$11.94 \pm 1.17^{a}{}_{B}$	$19.78 \pm 1.14^{b}{}_{B}$	$18.58 \pm 1.00^{b}{}_{B}$	$17.31 \pm 0.97^{ab}{}_{B}$	$17.28 \pm 1.43^{ab}{}_{B}$
CV_C	$6.95 \pm 0.70_{A}$	$7.36 \pm 1.46_{A}$	$7.54 \pm 0.88_{A}$	$7.63 \pm 0.25_{A}$	$7.94 \pm 1.22_{A}$
HSI_S	$1.55 \pm 0.10^{a}{}_{A}$	2.33 ± 0.05^{b}	2.00 ± 0.09^{ab}	$1.74 \pm 0.09^{a}{}_{A}$	$2.00 \pm 0.15^{ab}{}_{A}$
HSI_C	$2.19 \pm 0.04^{a}{}_{B}$	2.24 ± 0.06^{a}	2.22 ± 0.05^{a}	$2.37 \pm 0.06^{a}{}_{B}$	$2.65 \pm 0.05^{b}{}_{B}$
Ht_S	${}_{A}34.96 \pm 0.83^{b}$	${}_{A}33.69 \pm 0.40^{b}$	${}_{A}28.06 \pm 1.72^{a}$	${}_{A}26.38 \pm 0.48^{a}$	${}_{A}26.58 \pm 0.05^{a}$
Ht_C	${}_{B}47.00 \pm 0.32$	${}_{B}45.30 \pm 2.14$	${}_{B}44.23 \pm 2.57$	${}_{B}41.50 \pm 0.21$	${}_{B}43.07 \pm 2.70$
RBC_S	${}_{A}3.06 \pm 0.06^{b}$	${}_{A}2.86 \pm 0.02^{b}$	${}_{A}2.36 \pm 0.09^{a}$	${}_{A}2.17 \pm 0.01^{a}$	${}_{A}2.22 \pm 0.04^{a}$
RBC_C	${}_{B}4.36 \pm 0.05^{b}$	${}_{B}4.28 \pm 0.19^{b}$	${}_{B}3.94 \pm 0.04^{b}$	${}_{B}3.34 \pm 0.05^{a}$	${}_{B}3.45 \pm 0.09^{a}$
Hb_S	6.39 ± 0.08^{b}	6.31 ± 0.01^{ab}	6.08 ± 0.07^{a}	5.62 ± 0.04^{a}	5.46 ± 0.06^{a}
Hb_C	${}_{B}7.60 \pm 0.32^{b}$	${}_{B}6.70 \pm 0.21^{ab}$	${}_{B}6.60 \pm 0.15^{ab}$	${}_{B}5.90 \pm 0.17^{a}$	${}_{B}5.96 \pm 0.18^{a}$

Values are displayed as mean of triplicate groups ± SE. Means with different lowercase alphabets (a, b) within a row are significantly different ($P < 0.05$). Mean with different upper case alphabets (A, B) within similar parameter show the significant difference between snapper and cobia at each inclusion level of lupin meal ($P < 0.05$). CV: Coefficient variances, HSI: Hepatosomatic index, RBC: Red blood cell (x 10^{12}/l), Ht: Haematocrit (%) and Hb: Haemoglobin (g/dl).

The cobia fed the LKM315 and LKM420 diets had reduced RBC and Hb levels, while a dietary LKM of 210 g/kg caused negative effects on RBC and Hb levels of the snapper.

The dietary LKM also significantly affected the Ht of snapper, but not cobia (Table 4.3.5).

4.3.5 Amino acid profiles

Dietary LKM had no significant effects on the amino acid profiles in the muscle tissues of the snapper. Similar results also were observed in the cobia, except threonine, where cobia fed the highest level of LKM resulted in significantly lower threonine compared to those in the fish fed lower inclusion levels of LKM. There were no significant differences in the total essential amino acids (TEAA) and total non-essential amino acids (TNEAA) in both snapper and cobia fed the test diets. The TNEAA to TEAA ratio did not show any differences in the cobia, whereas this ratio was significantly lower in the snapper fed the LKM105 diet than that in the fish fed other diets (Table 4.3.6).

4.3.6 Histological evaluations

Both snapper and cobia fed high dietary LKM showed histopathological lesions in the livers. The livers of the cobia fed the highest LKM level showed more atrophic hepatocytes (Figure 4.3.2) than the fish fed lower levels of LKM, whereas increases in lipid droplet accumulation was observed in the livers of the snapper fed the LKM315 and LKM420, especially in the snapper fed the LKM420 diet (Figure 4.3.3). Cobia and snapper fed dietary LKM did not show any histopathological lesion in their distal intestines.

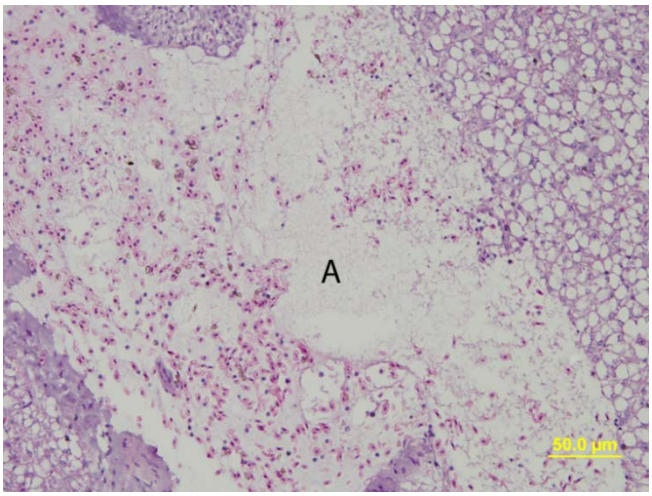


Figure 4.3.2 Liver section of cobia fed diet including 420 g/kg LKM showing the hepatocyte atrophy (A). Haematoxylin and eosin.

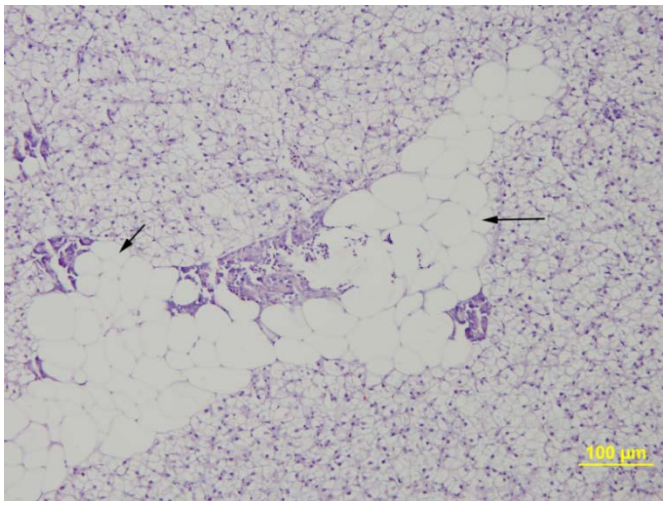


Figure 4.3.3 The increased hepatic lipid accumulation in snapper fed diet containing 420 g/kg LKM (arrow). Haematoxylin and eosin.

Table 4.3.6 Muscle amino acid profiles (g/100 g dry weight sample) of snapper and cobia fed test diets

Amino acids	LKM0		LKM105		LKM210		LKM315		LKM420		Pooled SE	
	Snapper	Cobia	Snapper	Cobia	Snapper	Cobia	Snapper	Cobia	Snapper	Cobia	Snapper	Cobia
Essential amino acids (EAAs)												
Arg	$_{B}$5.25	$_{A}$3.17	$_{B}$5.39	$_{A}$3.15	$_{B}$5.29	$_{A}$3.12	$_{B}$5.27	$_{A}$3.14	$_{B}$5.23	$_{A}$3.16	0.03	0.01
His	2.04	2.45	2.13	2.43	2.09	2.42	2.06	2.38	$_{A}$1.97	$_{B}$2.49	0.02	0.02
Ile	3.74	4.00	3.99	4.22	3.90	4.23	3.92	4.05	3.88	4.31	0.03	0.05
Leu	6.73	6.65	7.00	6.67	6.89	6.67	6.92	6.72	6.87	6.64	0.04	0.03
Lys	$_{A}$7.67	$_{B}$9.31	$_{A}$8.05	$_{B}$9.27	7.82	8.96	$_{A}$7.82	$_{B}$9.28	$_{A}$7.73	$_{B}$8.99	0.05	0.11
Met	$_{B}$2.64	$_{A}$1.99	$_{B}$2.74	$_{A}$1.90	$_{B}$2.71	$_{A}$1.92	$_{B}$2.73	$_{A}$1.89	$_{B}$2.70	$_{A}$2.10	0.02	0.04
Phe	3.41	3.25	3.54	3.27	3.48	3.27	3.50	3.26	3.44	3.26	0.02	0.01
Thr	$_{B}$3.96	$_{A}$2.47$_{c}$	$_{B}$4.09	$_{A}$2.42$_{ab}$	$_{B}$4.03	$_{A}$2.46$_{bc}$	$_{B}$4.02	$_{A}$2.46$_{bc}$	$_{B}$4.01	$_{A}$2.39$_{a}$	0.02	0.01
Val	4.04	4.00	4.26	4.01	4.14	4.04	4.16	4.01	4.13	4.02	0.03	0.02
TEAAs	$_{B}$39.49	$_{A}$37.27	$_{B}$41.20	$_{A}$37.36	$_{B}$40.37	$_{A}$37.09	$_{B}$40.39	$_{A}$37.21	$_{B}$39.96	$_{A}$37.03	0.23	0.13
Non-essential amino acids (NEAAs)												
Ala	$_{B}$5.19	$_{A}$4.52	$_{B}$5.30	$_{A}$4.71	$_{B}$5.25	$_{A}$4.58	$_{B}$5.24	$_{A}$4.75	5.21	4.81	0.02	0.13
Asp	$_{B}$9.09	$_{A}$6.14	$_{B}$9.43	$_{A}$6.17	$_{B}$9.38	$_{A}$6.16	$_{B}$9.50	$_{A}$6.11	$_{B}$9.34	$_{A}$6.25	0.06	0.02
Cys	$_{B}$1.02	$_{A}$0.65	$_{B}$1.02	$_{A}$0.65	$_{B}$1.01	$_{A}$0.65	$_{B}$1.01	$_{A}$0.65	$_{B}$1.00	$_{A}$0.66	0.01	0.01
Glu	$_{B}$12.25	$_{A}$9.42	$_{B}$12.78	$_{A}$9.38	$_{B}$12.79	$_{A}$9.39	$_{B}$12.81	$_{A}$9.39	$_{B}$12.74	$_{A}$9.37	0.08	0.01
Gly	3.96	4.03	4.02	4.01	3.95	4.04	3.95	4.05	3.90	4.04	0.02	0.01
Pro	3.33	3.86	3.44	3.85	3.46	3.84	3.42	3.79	3.35	3.81	0.03	0.01
Ser	$_{B}$3.71	$_{A}$3.03	$_{B}$3.80	$_{A}$2.99	$_{B}$3.77	$_{A}$3.09	$_{B}$3.78	$_{A}$3.03	$_{B}$3.75	$_{A}$3.10	0.02	0.02
Tau	0.08	0.11	0.08	0.10	0.11	0.11	0.12	0.10	0.14	0.11	0.01	0.003
Tyr	2.96	2.90	3.08	2.91	3.02	3.00	3.02	2.91	3.01	3.01	0.02	0.02
TNEEAs	$_{B}$41.58	$_{A}$34.66	$_{B}$42.94	$_{A}$34.79	$_{B}$42.73	$_{A}$35.53	$_{B}$42.85	$_{A}$34.77	$_{B}$42.44	$_{A}$35.17	0.22	0.14
TNEAAs/TEAAs	$_{B}$1.05^{ab}	$_{A}$0.93	$_{B}$1.04^{a}	$_{A}$0.93	1.06^{ab}	0.96	$_{B}$1.06^{ab}	$_{A}$0.93	1.07^{b}	0.95	0.003	0.01

Values are displayed as mean of triplicate groups. Means with different lowercase alphabets (a, b) within a row are significantly different ($P < 0.05$). Mean with different upper case alphabets (A, B) within similar parameter show the significant difference between snapper and cobia at each inclusion level of lupin meal ($P < 0.05$). TEAAs, Total essential amino acids (NEAAs); TNEAAs, Total non-essential amino acids (NEAAs).

4.4 EXPERIMENT 5: BENEFICIAL EFFECTS OF SELENIUM SUPPLEMENTATION ON COBIA FED LUPIN-BASED DIETS

4.4.1 Growth and feed utilisation performances

There were significant ($P < 0.05$) effects of dietary LKM, Se supplementation and their interaction on the FBW, SGR, FI and FCR of the cobia after 7 weeks of feeding (Table 4.4.1). In the absence of Se supplementation, cobia fed 315 g/kg LKM resulted in a reduction in FBW, SGR and FI, whereas FCR was increased at an inclusion level of 210 g/kg LKM. Fish fed lupin-based diets supplemented with 0.8 mg/kg Se achieved significantly higher FBW, SGR, FI and FCR than the fish fed lupin-based diets without Se supplementation.

Table 4.4.1 Growth and feed utilisation of cobia fed the test diets.

	FW (g/fish)	SGR (%/day)	FI (g/fish)	FCR	PR	ER	Survival (%)
Diets							
LP0	84.46^{cd}	3.11^{cd}	92.08^{c}	1.39^{a}	24.59^{d}	23.42	100
LP40	79.16^{c}	2.96^{c}	89.39^{c}	1.48^{b}	23.36^{c}	21.56	100
LP60	61.55^{a}	2.44^{a}	68.64^{a}	1.60^{c}	20.19^{a}	19.28	97.22
LP0Se	85.28^{d}	3.13^{d}	91.39^{c}	1.37^{a}	25.22^{d}	23.66	97.22
LP40Se	85.71^{d}	3.12^{d}	94.99^{c}	1.42^{a}	24.68^{d}	22.37	100
LP60Se	72.93^{b}	2.79^{b}	80.02^{b}	1.47^{b}	22.23^{b}	20.90	94.44
Pooled SE	2.14	0.06	2.25	0.02	0.43	0.38	0.84
Means of main effects of replacement level							
0	84.87	3.12	91.74	1.38	24.91	23.54^{C}	98.61
40	82.44	3.04	92.19	1.45	24.02	21.96^{B}	100
60	67.24	2.61	74.33	1.54	21.21	20.09^{A}	95.83
Means of main effects of dietary Se							
0	75.06	2.84	83.37	1.49	22.72	21.42^{X}	99.07
0.8	81.31	3.01	88.80	1.42	24.04	22.31^{Y}	97.22
Two-way ANOVA: P values							
Lupin	0.000	0.000	0.000	0.000	0.000	0.000	0.139
Se	0.000	0.000	0.000	0.000	0.000	0.002	0.271
Lupin x Se	0.002	0.001	0.003	0.002	0.011	0.084	0.723

Values are displayed as mean of triplicate groups. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significantly differences ($P < 0.05$) among all dietary treatments.

Means with different uppercase alphabets (A, B, C or X, Y) within a column indicate the significantly differences (P < 0.05) among means of the main effects of fishmeal replacement level and dietary Se supplementation, respectively. FW: Final weight (g), SGR: Specific growth rate, FI: Feed intake, FCR: Feed conversion ratio, PR: Protein retention and ER: Energy retention.

Reduced PR and ER were observed in the fish fed lupin-based diets without Se supplementation. There was a significant effect of the interaction between dietary LKM and Se supplementation on the PR, but not on ER. High survival rates were attained in all dietary treatments and were not significantly (P > 0.05) affected by dietary LKM, Se supplementation or their interaction (Table 4.4.1).

Table 4.4.2 Apparent digestibility coefficients (ADC %) and haematological parameters of cobia fed test diets.

	ADC of protein	ADC of energy	ADC of dry matter	Ht (%)	RBC	Hb
Diets						
LP0	87.80	75.04	67.17	42.15	4.24^{b}	7.16^{bc}
LP40	85.20	66.32	63.92	42.62	4.18^{b}	7.05^{b}
LP60	82.46	62.80	58.21	41.99	3.97^{a}	6.35^{a}
LP0Se	89.42	76.35	69.63	43.55	4.31^{b}	7.28^{c}
LP40Se	87.96	70.39	64.25	43.99	4.30^{b}	7.24^{bc}
LP60Se	85.11	66.17	60.79	43.64	4.24^{b}	7.13^{bc}
Pooled SE	0.59	1.21	0.95	0.31	0.03	0.33
Means of main effects of replacement level						
0	88.61^{C}	75.69^{C}	68.40^{C}	42.85	4.28	7.22
40	86.58^{B}	68.36^{B}	64.08^{B}	43.31	4.24	7.15
60	83.79^{A}	64.49^{A}	59.50^{A}	42.82	4.11	6.74
Means of main effects of dietary Se						
0	85.15^{X}	68.06^{X}	63.10^{X}	42.26^{X}	4.13	6.86
0.8	87.50^{Y}	70.97^{Y}	64.89^{Y}	43.73^{Y}	4.29	7.22
Two-way ANOVA: P values						
Lupin	0.000	0.000	0.000	0.744	0.001	0.000
Se	0.002	0.000	0.008	0.025	0.000	0.000
Lupin x Se	0.990	0.126	0.230	0.977	0.024	0.000

Values are displayed as mean of triplicate groups. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significantly differences (P < 0.05) among all dietary treatments. Means with different uppercase alphabets (A, B, C or X, Y) within a column indicate the significantly differences (P < 0.05) among means of the main effects of fishmeal replacement level and dietary Se supplementation, respectively. Ht: haematocrit, RBC: Red blood cell, Hb: Haemoglobin.

4.4.2 Digestibility

In the absence of Se supplementation, the cobia fed dietary LKM showed significantly lower nutrient digestibility than the fish fed the control diet. At each inclusion level of LKM, the digestibility of protein, energy and dry matter significantly improved when the diet was supplemented with Se. The interaction between dietary lupin and Se supplementation did not affect the protein, energy or dry matter digestibility of the cobia (Table 4.4.2).

4.4.3 Haematological parameters

The Ht was significantly ($P < 0.05$) affected by dietary Se supplementation, but not by dietary LKM levels. There were significant effects of dietary LKM level, Se supplementation and their interaction on the RBC and Hb of the cobia at the end of the feeding period. In the absence of Se supplementation, the RBC and Hb concentrations were significantly reduced in the cobia fed 315 g/kg LKM; however, the RBCs and Hb concentrations significantly increased in cobia fed 315 g/kg LKM supplemented with Se compared with the fish fed the same diet without Se supplementation (Table 4.4.2).

4.4.4 Proximate composition

Neither dietary LKM nor Se supplementation significantly ($P > 0.05$) affected the protein, dry matter and ash contents in the muscle tissues of cobia. However, dietary LKM, but not Se supplementation, significantly affected the lipid content and gross energy in the muscles of the cobia (Table 4.4.3). The muscle amino acid profiles did not show any significant differences among treatments (Table 4.4.4). There were no significant effects due to the interaction between dietary lupin levels and Se supplementation on the muscle composition or amino acid profiles of the cobia at the end of the feeding period. Dietary LKM level, Se supplementation and their interaction had significant effects on Se deposition in the cobia tissues. Regression analysis revealed positive linear relationships between dietary Se levels and tissue Se accumulation in cobia ($y = 0.177x + 0.1795$, $R^2 = 0.9585$ with $P < 0.001$ for the muscle Se, Figure 4.4.1 and $y = 0.5222x + 0.6685$, $R^2 = 0.9516$ with $P < 0.001$ for the liver Se, Figure 4.4.2).

Table 4.4.3 The muscle compositions (%) of cobia fed test diets.

	Protein	Lipid	Dry matter	Ash	Gross energy MJ/kg
Diets					
LP0	18.22	2.41	23.57	1.51	5.45
LP40	18.08	2.41	22.88	1.55	5.28
LP60	17.98	2.31	22.24	1.58	5.10
LP0Se	18.24	2.40	23.32	1.57	5.44
LP40Se	18.19	2.43	22.96	1.59	5.32
LP60Se	17.97	2.36	22.85	1.58	5.14
Pooled SE	0.05	0.01	0.19	0.01	0.04
Means of main effects of replacement level					
0	18.23	2.40^{B}	23.44	1.56	5.44^{B}
40	18.14	2.42^{B}	23.08	1.57	5.30^{B}
60	17.98	2.34^{A}	22.54	1.58	5.12^{A}
Means of main effects of dietary Se					
0	18.09	2.38	23.00	1.56	5.28
0.8	18.13	2.39	23.04	1.58	5.31
Two-way ANOVA: P values					
Lupin	0.152	0.000	0.090	0.567	0.001
Se	0.685	0.078	0.894	0.267	0.548
Lupin x Se	0.870	0.081	0.438	0.551	0.610

Values are displayed as mean of triplicate groups. Means with different lowercase alphabets (a, b, c, d) within a column indicate the significantly differences ($P < 0.05$) among all dietary treatments. Means with different uppercase alphabets (A, B, C) within a column indicate the significantly differences ($P < 0.05$) among means of the main effects of fishmeal replacement level.

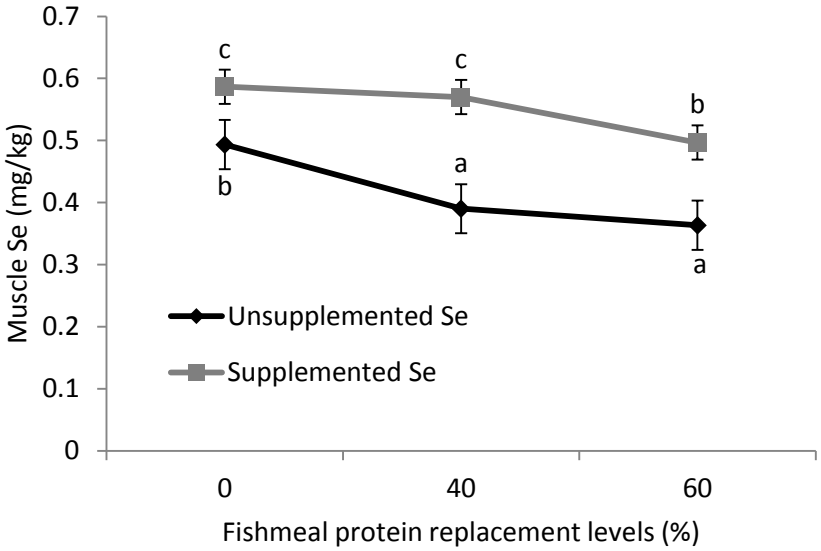


Figure 4.4.1 Mean of muscle Se concentrations in cobia fed diets containing various inclusion levels of lupin kernel meal with and without Se supplementation, mean with different letters are significantly different ($P < 0.05$).

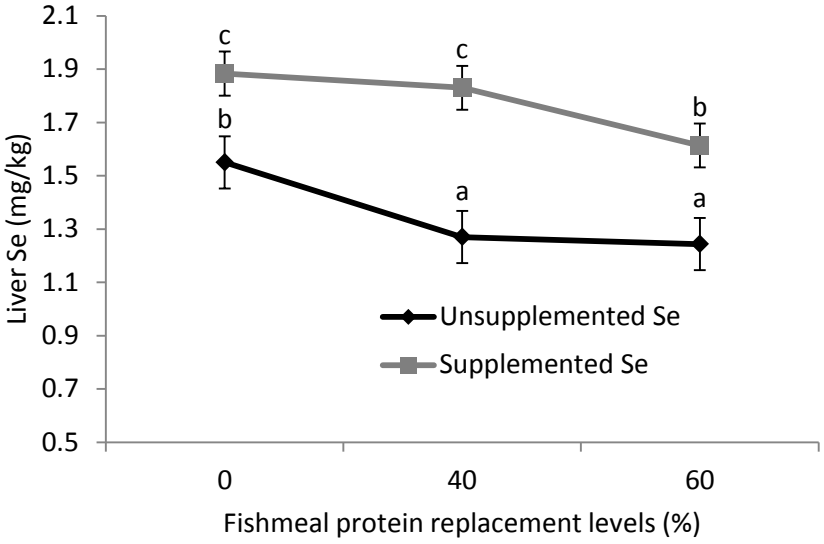


Figure 4.4.2 Mean of liver Se concentrations in cobia fed diets containing various inclusion levels of lupin kernel meal with and without Se supplementation, mean with different letters are significantly different ($P < 0.05$).

Table 4.4.4 Essential amino acid profiles (g/100 g dry weight sample) in muscle of cobia fed test diets

	Arg	His	Iso	Leu	Lys	Met	Phe	Thr	Tyr	Valine
Diets										
LP0	3.16	2.43	4.10	6.57	9.22	2.01	3.15	2.43	2.94	3.96
LP40	3.14	2.38	4.19	6.45	9.01	1.94	3.17	2.43	3.00	4.02
LP60	3.14	2.37	4.04	6.67	9.25	1.94	3.20	2.41	2.95	3.98
LP0 + Se	3.15	2.39	4.17	6.68	9.01	2.06	3.23	2.44	2.99	3.96
LP40 + Se	3.13	2.42	4.20	6.67	9.26	1.90	3.19	2.42	2.95	3.98
LP60 + Se	2.81	2.36	4.12	6.65	9.23	1.98	3.27	2.41	2.97	3.94
Pool SE	0.06	0.01	0.02	0.01	0.04	0.02	0.02	0.01	0.01	0.02
Two-way ANOVA: P values										
Lupin	0.39	0.25	0.15	0.37	0.43	0.17	0.60	0.13	0.84	0.67
Se	0.34	0.93	0.27	0.09	0.92	0.70	0.22	0.86	0.79	0.48
Lupin x Se	0.43	0.34	0.79	0.10	0.09	0.71	0.80	0.89	0.28	0.87

Values are displayed as mean of triplicate groups. Means with same letters within a column are not significantly different ($P > 0.05$). SEM: pool standard error of the mean.

CHAPTER 5. DISCUSSIONS

5.1. INTRODUCTION

Se, a trace element was first explored by Jons Jacob Berzelius in 1817 (Terry & Diamond, 2012). In periodic table of elements, Se is collocated between sulphur and tellurium in Group Iva, and has atomic number and weight of 34 and 78.971, respectively (Foster & Sumar, 1997). Se had been well known to be a toxic mineral for animals during 1930 – 1940s (Terry & Diamond, 2012) before its beneficially biological effect in preventing the hepatic necrosis in rats was discovered (Schwarz & Foltz, 1958). As both Se and sulphur have similar chemical properties; Se can easily replace sulphur to form different Se compounds (Barceloux, 1999). The incorporation of Se into proteins was established by Rotruck *et al.* (1973), and more than ten selenoproteins in forms of GPx and iodothyronine deiodinases have been identified (Luten *et al.*, 2008).

The necessity of Se in maintaining normal growth and physiological functions have been demonstrated in fish due to its important role as a cofactor in GPx enzyme, protecting cell membranes against oxidative damage (Lin & Shiau, 2005; Watanabe *et al.*, 1997). The deficiency of Se can lead to reduced growth, feed utilisation (Lin & Shiau, 2005; Liu *et al.*, 2010) and health status (Liu *et al.*, 2010) in farmed fish. Whereas fish fed elevated dietary Se levels results in reduced feed utilisation and adverse effects on physiological performance and impaired histology (Le & Fotedar, 2014b; Lee *et al.*, 2008; Lee *et al.*, 2010; Tashjian *et al.*, 2006; Teh *et al.*, 2004). Due to the narrow gap between deficiency, optimality and toxicity of Se level, it is imperative to find out the exact dietary requirement, bioavailability and the source of dietary Se for any aquatic species.

Due to the complexity of the Se availabilities in feedstuffs, past investigations have used casein as a sole protein source in the purified or semi-purified diets to quantify dietary Se requirement for fish (Hilton *et al.*, 1980; Lee *et al.*, 2008; Lin, 2014; Lin & Shiau, 2005; Liu *et al.*, 2010). However, fish fed these diets generally requires relatively lower dietary Se levels than for the fish fed commercial or formulated basal diets (Table 5.1.1). The high cost of casein and the reduced growth in fish fed casein-based diets than fishmeal-based diets make casein-based diet a non-practical under commercial culture environment. Though, Watanabe *et al.* (1997) stated that the Se concentration in fishmeal could provide adequate Se to meet the Se demand in fish but due to its significantly lower digestibility than other organic Se forms (Bell & Cowey, 1989; Le &

Fotedar, 2014a; Watanabe *et al.*, 1997), fish fed fishmeal or plant-based diets may require additional dietary Se to meet the nutritional requirement of the host species (Abdel-Tawwab *et al.*, 2007; Ilham *et al.*, 2016a; Le & Fotedar, 2013; Zhu *et al.*, 2012). Besides, the changes in dietary formulations have resulted in alteration of ingredients fed to fish (Read *et al.*, 2014); thus, dietary Se requirement may need to be re-evaluated due to changeability in the light of bioavailability of Se from various protein sources.

Table 5.1.1 Dietary Se requirements quantified for fish using different diet formulations

Name	Protein sources	Se source	Optimum Se mg/kg	References
Rainbow trout *Oncorhynchus mykiss*	Casein & Torula yeast	Selenite	0.15 - 0.38	Hilton *et al.* (1980)
Channel catfish *Ictalurus punctatus*	Casein	Selenite	0.25	Gatlin and Wilson (1984)
Grouper	Casein	Se-Met	0.7	Lin and Shiau (2005)
Black seabream *Acathopagrus schlegeli*	Casein	Selenite	0.21	Lee *et al.* (2008)
Cobia *Rachycentron canadum*	Casein	Se-Met	0.78 – 0.81	Liu *et al.* (2010)
Gibel carp *Carassius auratus gibelio*	Casein	Se-Met	1.18	Han *et al.* (2011)
African catfish *Clarias gariepinus*	Soybean	Se-yeast	3.67	Abdel-Tawwab *et al.* (2007)
Largemouth *Micropterus salmoide*	Fishmeal & Soybean	Selenite	1.60 – 1.80	Zhu *et al.* (2012)
Yellowtail kingfish *Seriola lalandi*	Commercial diet	Se-yeast	5.56	Le and Fotedar (2013)
Yellowtail kingfish *Seriola lalandi*	Fishmeal	Se-Met	4.91	Le and Fotedar (2014b)

In nature, carnivorous marine fish such as snapper and cobia need high dietary protein supply to provide sufficient amino acids and nitrogen for the synthesis of essential amino acids and other biological compounds (Fraser & Davies, 2009; Tacon & Metian, 2008). Fishmeal has been widely incorporated in formulated diets for marine fish species since its high protein content, well-balanced essential amino acids and high nutrient digestibility (Gatlin *et al.*, 2007; Hertrampf & Piedad-Pascual, 2000; Olsen & Hasan, 2012). However, high demand and unstable supply of fishmeal have led to investigations to find alternative protein sources to fishmeal (Olsen & Hasan, 2012; Tacon & Metian,

2008). At present, various protein sources with variable inclusion levels of plant-derived ingredients have already been incorporated into formulated feeds for marine species.

Lupin meals such as, narrow-leafed lupin, white lupin and yellow lupin have been shown to be a potentially alternative protein source to fishmeal in aqua-feeds due to their high protein contents, reasonable prices and easy availabilities (Pereira & Oliva-Teles, 2004; Salini & Adams, 2014). The beneficial effects of lupin kernel meal (LKM) have been demonstrated in rainbow trout (Borquez *et al.*, 2011), gilthead seabream (Pereira & Oliva-Teles, 2004; Robaina *et al.*, 1995), Atlatic salmon (Carter & Hauler, 2000) and barramundi (Ilham *et al.*, 2016a) (more detailed in Table 2.2.5). However, adverse effects in growth and feed efficiency as well as histopathological damages induced by imbalanced amino acid profiles and anti-nutritional factors in lupins have been revealed in fish fed high inclusion levels of lupin (Borquez *et al.*, 2011; Glencross *et al.*, 2004b; Refstie *et al.*, 2006).

Besides, the increasing inclusion level of plant protein sources in aqua-feeds has also put an increasing pressure on the dietary mineral requirements including zinc and Se due to their lower concentration in plant-derived ingredients (Antony Jesu Prabhu *et al.*, 2016; Welker *et al.*, 2016). Besides, the interaction between Se and other minerals such as copper, sulphur, mercury (Watanabe *et al.*, 1997) and vitamin E (Le *et al.*, 2014b; Lin & Shiau, 2009) alters the bioavailability of Se in fish, making investigation on Se requirement more complex. The anti-nutritional factors presented in plant-derived ingredients can also interact with minerals, reducing their bioavailabilities (Antony Jesu Prabhu *et al.*, 2016). Thus, fish fed plant-based diets may require mineral fortification to maximise their potential growth at higher concentration than levels optimised for purified or semi-purified diets (Barrows *et al.*, 2008; Barrows *et al.*, 2010; Read *et al.*, 2014; Welker *et al.*, 2016).

Compared with snapper, cobia have relatively faster growth rate (Table 5.1.2). As faster growing fish have higher metabolic rates to provide sufficient energy to maximise their growth potential (DeVries & Eastman, 1981). The lack of air-bladder in cobia results in higher feed consumption to meet their energy requirement for metabolism, as seen in trouts (Luísa Maria Pinheiro Valente *et al.*, 1998). Therefore, it could be hypothesised that cobia requires higher feed intake than snapper due to the differences in their capacities for digestion, growth rates, as reported in rainbow trout and barramundi

(Glencross *et al.*, 2011), rainbow trout and Atlantic salmon (Glencross *et al.*, 2004a; Refstie *et al.*, 2000) and between rainbow trout and snapper (Glencross *et al.*, 2004c).

Table 5.1.2 A comparison on specific growth rate (SGR) of snapper and cobia

Snapper			Cobia		
FM in the control diet	SGR	References	FM in the control diet	SGR	References
900	1.02	Booth *et al.* (2005)	560	2.03	Luo *et al.* (2012)
490	0.97	Booth *et al.* (2005)	494	2.39	Trushenski *et al.* (2011)
640	0.78	Quartararo *et al.* (1998a)	558	3.6	Romarheim *et al.* (2008)
420	1.69	Glencross *et al.* (2003b)	586	4.17	Lunger *et al.* (2007)
600	0.96	Booth *et al.* (2012)	253	2.50	Salze *et al.* (2010)
590	1.67	Glencross *et al.* (2003b)	455	3.29	Watson *et al.* (2014b)
Commercial diet	1.01	Booth *et al.* (2012)	230	3.49	Ren *et al.* (2014)
			Commercial diet	3.54	Weirich *et al.* (2010)

FM: fishmeal, SGR: specific growth rate.

5.2. EFFECTS OF DIETARY ORGANIC SELENIUM SUPPLEMENTATION IN SNAPPER

In Australia, the snapper industry is relatively small due to the regulatory restrictions in farming of the species in coastal cages, the lack of a dedicated commercial diet, confusion surrounding the nomenclature of the species and slower growth rates. The species has otherwise shown positive attributes for farming including high market price and important recreational and commercial fisheries (Booth *et al.*, 2005; Fielder *et al.*, 2001). Currently, the snapper farming relies on the commercial feeds of other marine fish (Booth *et al.*, 2012). There are significant inconsistencies in the proximate composition of the reference diets tested for snapper (Booth *et al.*, 2005; Booth *et al.*, 2012; Booth *et al.*, 2006; Glencross *et al.*, 2003b; Glencross *et al.*, 2003c; Glencross *et al.*, 2004c; Quartararo *et al.*, 1998a) in which crude protein and lipids ranged from 50 - 70 %, and 6.8 – 16.4 %, respectively (Table 5.3.1), leading to a lack of nutrition knowledge of macro and micro nutrient requirements, including Se.

In the current study, the protein, lipid, carbohydrate and gross energy contents in the basal and reconstituted commercial diets were 465.6, 115.0, 246.8 g/kg and 20.70 MJ/kg and 495.2, 16.20, 331.6 g/kg and 21.64 MJ/kg, respectively, and no differences were observed in the growth of snapper due to the diet types. The growth rate of snapper in the present study was also similar to that of snapper fed high dietary protein levels as described by Booth *et al.* (2012), Quartararo *et al.* (1998a) and Glencross *et al.* (2003b). Quartararo *et al.* (1998b) also found no difference in the growth performance of Australian snapper fed a commercial diet containing 350 and 510 g/kg crude protein due to the protein-sparing effect (Chou *et al.*, 2001; Meyer & Fracalossi, 2004; Quartararo *et al.*, 1998b). The improvement in protein utilisation and reduced nitrogen excretion in fish has been evidenced by supplying non-protein energy sources to meet energy requirements (Cho & Bureau, 2001; Kaushik & Seiliez, 2010). Protein, lipids and carbohydrates are the major energy sources in which lipids are efficiently utilised, whereas carbohydrates, a less costly energy source, is inefficiently utilised in marine carnivorous fish (de la Higuera, 2001). A marine carnivore but slower-growing fish, such as snapper, may be capable of digesting non-protein energy sources, such as carbohydrates, to meet their energy needs, as shown in rainbow trout (Cho, 1992) and gilthead seabream (Vergara *et al.*, 1996). In our study, all basal diets had relatively lower fat and protein contents, but relatively higher carbohydrates than reconstituted commercial diets. However, snapper did not show any differences in growth performances, whereas the fish fed BS diets attained higher feed utilisation efficiency than the fish fed the CD diets.

Table 5.2.1 Overviews the proximate composition of control diets fed to Australian snapper.

Name	Crude protein (g/kg)	Crude lipid (g/kg)	Crude ash (g/kg)	Gross energy (MJ/kg)	References
Control diet	539	78	146	18.1	Quartararo *et al.* (1998a)
Control diet	517	68	114	20.8	Glencross *et al.* (2003c)
Control diet	519	164	124	22.1	Glencross and Hawkins (2004)
Control diet	680	174	129	23.2	Booth *et al.* (2006)
Control diet	700	141	111	20.9	Booth *et al.* (2005)
Atlantic salmon feed	520	n/a	n/a	18.0	Booth *et al.* (2008)
Barramundi feed	519	135	105	25.0	Booth *et al.* (2012)

In the current study, a commercial barramundi diet was used as a reference diet due to the unavailability of a commercial feed for Australian snapper. The difference between

feed-production processes used in formulating the basal diet in the laboratory and the commercial diet can result in different physical and chemical characteristics, such as water stability and durability, pellet hardness and nutrient bioavailability, consequently changing feed intake and utilisation in fish (Booth *et al.*, 2012; de la Higuera, 2001). Therefore, the commercial diet in our current study was ground and re-pelleted following the same methods used for the basal diet to produce test diets with similar physical characteristics. Snapper fed test diets did not show any difference in FI, except for the lower FI in fish fed basal diet including 2.77 mg/kg Se, due to the toxicity of Se in this diet as observed in other similar studies (Gatlin & Wilson, 1984; Le & Fotedar, 2014b).

Past research has shown that certain micronutrients, including Se, have an ability to compensate for lower dietary protein. Previous research quantifying the dietary requirements for Se in grouper (Lin, 2014; Lin & Shiau, 2005), black seabream (Lee *et al.*, 2008), cobia (Liu *et al.*, 2010), gibel carp (Han *et al.*, 2011), largemouth bass (Zhu *et al.*, 2012) and yellowtail kingfish (Le & Fotedar, 2013) obtained levels of 0.6 – 0.98, 0.21, 0.78, 1.18, 1.60-1.85 and 5.35 mg/kg Se, respectively. The different Se requirements of various species may relate to physiological differences, waterborne Se levels (Hilton *et al.*, 1980), the bioavailability of various selenium forms (Le & Fotedar, 2014a; Lin, 2014; Wang *et al.*, 2007) and the lack of consistency in the use of protein ingredients. Aside from species dependence, the faster growth rate demands higher dietary Se, for example, in cobia (Liu *et al.*, 2010) and yellowtail kingfish (Le & Fotedar, 2013).

The Australian snapper has relatively slower growth rates than cobia and yellowtail kingfish, suggesting that the dietary Se requirement could be lower in snapper, as is now evident in the current research: dietary Se concentrations from 0.92 to 2.59 mg/kg had no significant effect on growth performance in snapper, whereas fish fed diet containing 2.77 mg/kg Se exhibited growth reduction. However, the response of snapper to dietary Se was also influenced by the dietary protein level as represented by either basal or reconstituted barramundi commercial diet: snapper fed dietary Se of only 1.76 mg/kg in the commercial diet displayed lower growth rates and feed utilisation efficiency with higher coefficient of variation, whereas this concentration in the basal diet was 2.77 mg/kg Se. Clearly, in the absence of Se supplementation, the dietary Se requirement of juvenile Australian snapper was met by natural Se present in other protein sources, such as fishmeal and soybean meal, in the basal and reconstituted commercial diets, and the Se threshold level in snapper was also affected by the type of the feed used.

Dietary nutrients have a direct relationship with nutrient accumulation in the tissues of aquatic species (Espe, 2008). For example, cobia fed excessively high dietary lipids displayed reduced protein and increased lipid levels in the whole body and liver tissues (Wang *et al.*, 2005). A low-protein diet can result in a deficiency of essential nutrients (Fraser & Davies, 2009; Kaushik & Seiliez, 2010), which can have an impact on protein synthesis and deposition (de la Higuera, 2001). In our study, the BS diet contained lower crude protein and lipid than the CD diet; however, the diet types had no effects on the proximate composition of snapper, except slight changes in the dry matter and gross energy in the body of snapper fed BS diet. Similar results were obtained in other marine species, where a wide range of protein and lipid diets were used (Craig *et al.*, 2006; Morais *et al.*, 2001). In addition, the reduced dry matter and gross energy in the body of snapper fed the BS diet supplemented with 1.0 mg/kg of Se could be due to the impact of high dietary Se levels, similar to results obtained in largemouth bass (Zhu *et al.*, 2012). Regardless of the diet types, in the current study, snapper fed diet with 1.0 mg/kg Se supplementation resulted in higher hepatic lipid concentration than the fish fed lower inclusion levels of Se, which may be associated with abnormal lipid metabolism in hepatopancreas caused by toxic effects of Se, as reported in largemouth bass (Zhu *et al.*, 2012) and sacramento splittail (Teh *et al.*, 2004).

Abdel-Tawwab *et al.* (2007) indicated that feeding African catfish a plant-based diet containing 5.54 mg/kg Se could cause toxic effects resulting in dysfunctioning of liver and kidney; however, a total dietary Se of 8.5 mg/kg in the fishmeal-based diet had no negative effects in this species (Schram *et al.*, 2008). Excessively high dietary Se levels have been linked to histological lesions in the liver tissues of yellowtail kingfish (Le & Fotedar, 2014b), green sturgeon *Acipenser medirostris*, white sturgeon (De Riu *et al.*, 2014) and green sunfish (Sorensen *et al.*, 1984). The liver of green sunfish with Se levels of 7.0 and 21.4 mg/kg dry weight, respectively, showed lymphocyte infiltration and an increase in lipid droplets relative to the fish containing 1.3 mg/kg Se in the liver (Sorensen *et al.*, 1984). The yellowtail kingfish exhibited atrophic hepatocytes in the liver with an Se concentration of 20.82 mg/kg dry weight (Le & Fotedar, 2014b). Glycogen depletion and vacuolar degeneration were observed in the livers of both green and white sturgeon fed dietary Se concentrations of 19.7, 40.1 and 77.7 mg/kg (De Riu *et al.*, 2014). Consistent with the histological changes in the livers of juvenile sacramento splittail exposed to high dietary Se concentrations (Teh *et al.*, 2004), the dietary Se levels of 2.59 and 2.77 mg/kg caused histopathological alterations, resulting in moderate lipid droplet deposition and severe fatty vacuolar degeneration in the livers

of snapper fed BS diet supplemented with 1.0 mg/kg Se. The cell necrosis in hepatocytes can be explained by the gradual deterioration in synthesis of new structural and metabolic component of the cell to restore the damages caused by toxic effects of Se, resulting in cell death (Teh *et al.*, 2004). Besides, glycogen depletion induced by increasing glycogenolysis may also cause single cell necrosis and macrophage aggregates in liver. Meanwhile, the lipid vacuolar degenerations in livers may be results of the changing in protein turnover and lipid metabolism caused by Se toxicity, consequently, resulting in incapacitation of liver in metabolism and excretion of biochemicals (Teh *et al.*, 2004).

The interactions between dietary micronutrients and the nature of dietary protein ingredients can change the absorption and metabolism of nutrients (Read *et al.*, 2014). In the common carp, feeding a low-protein diet (250 g/kg) with magnesium (Mg) supplementation resulted in significantly higher PER than feeding a higher protein diet (440 g/kg) at the same level of Mg supplementation (Dabrowska *et al.*, 1991). Increased protein utilisation and protein metabolic enzyme activity have also been demonstrated in African catfish fed a plant-based diet with organic Se supplementation (Abdel-Tawwab *et al.*, 2007). Similarly, the beneficial effect of dietary Se supplementation to improve protein digestibility have been reported in barramundi (Ilham *et al.*, 2016a), pigs (Chaudhary *et al.*, 2010; Tian *et al.*, 2006) and sheep (Shi *et al.*, 2011). In the current study, Australian snapper fed BS diet attained higher PER and EER than fish fed CD diet containing 0.92 mg/kg Se. This might be attributable to the increase in microbial activity, the activity of digestive enzymes in the protein degradation process stimulated by dietary Se, as reported in other animals (Chaudhary *et al.*, 2010; Shi *et al.*, 2011).

5.3. EFFECTS OF DIETARY SELENIUM SUPPLEMENTATION IN COBIA FED COMMERCIALLY AVAILABLE DIET

The insignificant amount of Se in casein allows nutritionists to use lowest-Se control diet to overcome the complication of the Se availabilities in feed ingredients. However, fish fed purified or semi-purified diets have reduced growth and feed intakes than the fish fed fishmeal-based diets (Hertrampf & Piedad-Pascual, 2000), thus, making the evaluation of dietary Se requirement challenging and meaningless. Meanwhile, the variability in the inclusion level of various protein sources in any commercial diet possess variations in the bioavailability of Se due to the different Se absorption and metabolic pathways adapted by differently sourced Se. These variations in the bioavailability of different

commercial marine fish diets alter the nutritional requirement of the Se by the host fish. The Se requirement is further complicated by the inherent growth abilities and physiological mechanisms of various species (Lin & Shiau, 2005; Liu *et al.*, 2010).

Based on the highest SGR, Liu *et al.* (2010) recommended an optimum dietary Se level for the juvenile cobia fed purified diet with protein sourced casein, to be 0.788 mg/kg, however, in the current study, juvenile cobia fed a commercially available diet required dietary Se at a higher concentration of 2.32 mg/kg to maximise their growth performances. The highest SGR of in the current study (3.46) was considerably higher than the SGR of cobia (2.62) reported by Liu *et al.* (2010). Similarly, in rainbow trout fed semi-purified diet showed relatively lower Se requirement, ranging from 0.15 to 0.38 mg/kg (Hilton *et al.*, 1980), whereas, the fish fed a commercial trout diet required higher dietary Se concentration (> 0.8 mg/kg) to improve Se accumulation and health status, especially under the stress conditions (KÜÇÜKbay *et al.*, 2009; Rider *et al.*, 2009). The higher dietary Se level required for cobia have been linked to the higher SGR in cobia (Liu *et al.*, 2010) than in grouper, rainbow trout, channel catfish and black seabream. As faster growing fish requires higher metabolic rate to provide sufficient energy to maximise the growth potential (DeVries & Eastman, 1981), thus, need to uptake more nutrients, including Se. Cobia fed a commercial diet showed considerably higher SGR than reported by Liu *et al.* (2010) using purified diet, pointing towards the fact that a faster growing fish may have higher dietary Se requirement than the fish having slower growth rates. However, this hypothesis needs further evidence.

Table 5.3.1 Regression between dietary Se level and tested parameters in juvenile cobia

Parameters	Equation	R^2	P-value
FBW	$Y = -83516X^2 + 37.634X + 51.205$	0.8012	< 0.001
FI	$Y = -12.5304X^2 + 58.2942X + 37.1282$	0.6966	< 0.001
RBC	$Y = -0.2218X^2 + 0.9292X + 3.0009$	0.7510	< 0.001
Hb	$Y = -0.6767X^2 + 2.8072X + 4.1511$	0.5825	0.001
GPx	$Y = -1.9422X^2 + 24.1984X + 31.2451$	0.9130	< 0.001
TGC	$Y = -0.0076X^2 + 0.3543 + 0.9503$	0.6749	< 0.001

FBW final body weight; FI feed intake; RBC red blood cells; Hb haemoglobin; GPx glutathione peroxidase; TGC thermal growth coefficient.

Other investigations have also indicated higher Se levels required for fish fed formulated diets (Abdel-Tawwab *et al.*, 2007; Le & Fotedar, 2013; Zhu *et al.*, 2012) than for the fish using purified or semi-purified diets (Hilton *et al.*, 1980; Lee *et al.*, 2008; Lin, 2014; Lin

& Shiau, 2005; Liu *et al.*, 2010). Besides, the cobia fed a commercial diet containing 1.15 mg/kg Se showed symptoms of Se deficiency, including reduced growth, accumulations of Se in the tissues and physiological changes, which are consistent with yellowtail kingfish (Le & Fotedar, 2013, 2014b), barramundi (Ilham *et al.*, 2016a), African catfish (Abdel-Tawwab *et al.*, 2007) and largemouth (Zhu *et al.*, 2012) fed fishmeal or plant-based diets.

In contracdiction to the studies of yellowtail kingfish (Le & Fotedar, 2014b), largemouth (Zhu *et al.*, 2012) and cobia in the current study, Liu et al. (2010) showed the increases in protein and lipid concentration in the whole-body of cobia with the increase in dietary Se. However, cobia fed 3.14 mg/kg Se diet resulted in significantly higher hepatic lipid accumulation than the fish fed lower dietary Se levels, which is associated with the abnormal lipid metabolism in hepatopancreas caused by toxic effects of Se, as reported in largemouth bass (Zhu *et al.*, 2012) and sacramento splittail (Teh *et al.*, 2004).

In the current study, tissue Se accumulations showed a positive linear relationship with dietary Se level, similar to those reported in other fish species (Abdel-Tawwab *et al.*, 2007; Han *et al.*, 2011; Jaramillo *et al.*, 2009; Le & Fotedar, 2014b; Lin & Shiau, 2005; Liu *et al.*, 2010), thus, the levels of Se in tissues can serve as bio-marker for dietary Se exposure. One of the major functions of liver is to metabolise and uptake and redistribution of Se in the body (Hinton *et al.*, 2008). It also controls the excretion or other processing of Se (Burk & Hill, 2009), thus, resulting in significantly higher hepatic Se concentrations than other tissues, as seen in yellowtail kingfish (Le & Fotedar, 2014b), grouper (Lin & Shiau, 2005), gibel carp (Han *et al.*, 2011) and in cobia in the current study. However, the reduction in liver and muscle Se ratio from 2.77:1 to 1.73:1, corresponding with increasing dietary Se level from 1.15 to 3.14 mg/kg in the present study indicated the higher potential for Se accumulation in muscle than hepatic tissue. This again could be linked to higher inherent growth rate of cobia.

As Ht level has a strong relationship with the internal environment in fish (Roche & Boge, 1996), and play important roles with respiratory activity, thus, the reduced Ht may interfere the respiratory capacity, resulting in the metabolic stress, consequently, impairing the fish health (Lemly, 1993). The reduction in Ht has been reported in yellowtail kingfish fed elevated Se level (Le & Fotedar, 2014b), similar to the current study. Similar to Ht, the RBC and Hb concentrations can be indicators of oxidative stress and toxicological impacts in fish (Hao *et al.*, 2014; Kiron *et al.*, 2004; Sharma *et al.*,

2014). In the current study, cobia fed the highest level of Se showed reduced RBC and Hb values, which could be attributed to the restriction in RBC production and Hb synthesis caused by the toxic effects of Se, similar to loach *Paramisgurnus dabryanus* (Hao *et al.*, 2014). As RBC is responsible for the Hb synthesis and transportation of oxygen and carbon dioxide in the blood (Olugbemi *et al.*, 2010), the increased RBC and Hb concentrations in cobia fed 1.93 mg/kg Se diet indicated the enhancement in fish health induced by dietary supplemental Se. The increased MCV or MCHC values have been linked to the swelling of RBC induced by the toxicity stress (Saravanan *et al.*, 2011; Wepener *et al.*, 1992), however, in the current study, dietary Se did not influence the shape of RBC, resulting in no change in MCV and MCHC of cobia.

The GPx has been used to evaluate the nutritional status of Se since its important roles in preventing oxidative stress by catalysing crucial reactions to reduce lipid peroxidation in the liver tissues (Lin & Shiau, 2005; Rotruck *et al.*, 1973; Watanabe *et al.*, 1997). In the present study, the cobia fed a commercial diet containing 1.15 mg/kg Se resulted in the lowest GPx activity, which is consistent with barramundi (Ilham *et al.*, 2016a), yellowtail kingfish (Le & Fotedar, 2014b) and grouper (Lin, 2014; Lin & Shiau, 2005) fed Se-deficient diets. In animals, the selenoproteins are regulated at ribosomal levels, thus, deficiency of Se supply tend to increase the Se required for the synthesis of essential selenoproteins, which are important for survival rather than for GPx enzyme (Burk & Hill, 1993), consequently, decreasing the GPx activity. In the current study, the GPx achieved a plateau in the cobia fed dietary Se of between 1.52 and 2.29 mg/kg, but increased in fish fed 2.71 mg/kg Se diet and above this level, as reported in yellowtail kingfish (Le & Fotedar, 2014b). The increased GPx activity in cobia fed elevated Se levels could be attributable to the increase in liver lipid peroxidation caused by Se toxicity, similar to reported in mallard ducks *Anas platyrhynchos* (Hoffman *et al.*, 1992).

Dietary Se supplementation had no effects on VSI, CF and CV of cobia, similar to largemouth bass (Zhu *et al.*, 2012). Hilton *et al.* (1980) and Han *et al.* (2011) showed that no relation exists between HSI and dietary Se level in rainbow trout and gibel carp, respectively. However, cobia fed 3.14 mg/kg Se diet resulted in lower HSI than the fish fed lower dietary Se levels, supported in yellowtail king fish (Le & Fotedar, 2014b), largemouth bass (Zhu *et al.*, 2012) and white sturgeon (Tashjian *et al.*, 2006). The smaller HSI in fish could be due to the atrophic hepatocytes induced by toxic effects of Se, as seen in white sturgeon (Tashjian *et al.*, 2006) and yellowtail king fish (Le & Fotedar, 2014b).

The liver serves as both target organ for chemical toxicity and defensive organ due to its important functions in synthesis, metabolism and redistribution of nutrients, as well as production and excretion of bile (Hinton *et al.*, 2008) and is associated with the histopathological and physiological alterations under toxic stress (Hinton *et al.*, 2008). The hepatic atrophy, macrophage aggregate, glycogen depletion and vacuolar degeneration have been observed in tissues of fish fed excessively dietary Se levels (De Riu *et al.*, 2014; Le & Fotedar, 2014b; Lee *et al.*, 2010; Tashjian *et al.*, 2006; Teh *et al.*, 2004). In the current study, cobia fed 3.14 mg/kg Se showed the necrotic hepatocytes and dilation of bile ducts, similar to the histological lesions in liver of white sturgeon after fed high Se (De Riu *et al.*, 2014; Tashjian *et al.*, 2006). The energetically vulnerable status in white sturgeon is attributable to fast growth rate in the species (De Riu *et al.*, 2014). In excessively Se supply, the triselenium linkage (Se-Se-Se) or a selenotrisulphide linkage (S-Se-S) is formulated during the protein synthesis since the erroneous substitution of Se for sulphur, instead of disulphide S-S linkages which have key roles for the normal tertiary structure of protein molecules, resulting in the protein dysfunction (Maier & Knight, 1994) and oxidative stress (De Riu *et al.*, 2014).

Compared to cobia, supplemental Se to either CD or BS diets had no effects on growth, feed intake and FCR of snapper (Table 5.4.2). Similarly, rainbow trout cultured in low density or under practical condition did not show any effects on growth and feed utilisation after being fed a CD or BS diets supplemented with either inorganic Se or organic Se forms (KÜÇÜKbay *et al.*, 2009; Rider *et al.*, 2009) due to the relatively low Se concentrations required for rainbow trout, as quantified by Hilton *et al.* (1980). In contrast, dietary Se supplementation had beneficial effects on growth and feed efficiency in cobia fed commercial diet, similar to those observed in yellowtail kingfish (Le & Fotedar, 2013) and largemouth (Zhu *et al.*, 2012). Owning to the species-dependant fast growth rates, higher dosage of nutrients including Se needs to be provided to satiate their growth requirement, resulting higher Se requirement in cobia than snapper.

Table 5.3.2 Performances of snapper and cobia fed Se-supplemented diets

Species	Growth	Survival	Feed intake	FCR	Proximate composition	RBC	Hb	GPx
Snapper	-	-	-	-	-	N/A	N/A	N/A
Cobia	+	-	+	-	-	+	+	+

FCR feed conversion ratio; RBC red blood cells; Hb haemoglobin; GPx glutathione peroxidase activity; N/A data not available; - none effect; + positive effect.

In this study, cobia fed 3.14 mg/kg Se diet showed signs of Se toxicity, including reduced growth, feed intake, RBC and Hb levels as well as histological alterations in liver tissues, whereas the threshold toxic levels of Se in snapper fed CD or BS diets were 1.76 and 2.77 mg/kg, respectively (Table 5.4.3). These levels are relatively lower than those previously quantified in black seabream (Lee *et al.*, 2008), olive flounder (Lee *et al.*, 2010), yellowtail kingfish (Le & Fotedar, 2014b) and white sturgeon (Tashjian *et al.*, 2006), but higher than the Se toxic levels determined for rainbow trout (Hilton *et al.*, 1980) and loach (Hao *et al.*, 2014). In fish, the toxic dietary Se level is species-dependant, and is also affected by the exposure period, Se forms and life stages of the host animal (Hao *et al.*, 2014; Lemly, 2002a; Teh *et al.*, 2004). Additionally, due to multiple physiological functions of liver, fish may show different pathways in response to toxic damages (Hinton *et al.*, 2008), which could be a reason explaining the variability of Se toxicity among these studies.

Table 5.3.3 Signs of Se toxicity in snapper and cobia

Parameter	Snapper	Cobia
	Signs of Se toxicity	Signs of Se toxicity
Growth	Reduced growth	Reduction in SGR, WG
Feed utilisation	Increased FCR and reduction in PER, EER, PR and ER	Reduced feed intake
Proximate composition	Reduced dry matter and gross energy in body	No record
Liver lipid	Increased hepatic lipid	Increased hepatic lipid
Somatic indices	Increased CV	Reduced HSI
Haematological responses	N/A	Reduced Ht, RBC and Hb
Histological responses	Increase lipid droplet accumulation in liver tissue	Dilation of bile duct and necrotic hepatocytes in liver tissue

SGR specific growth rate; WG weight gain; FCR feed conversion ratio, PER protein efficiency ratio; EER energy efficiency ratio; PR protein retention; ER energy retention; CV coefficient of variances; RBC red blood cells; Hb haemoglobin; HSI hepatosomatic index; N/A not available.

5.4. EFFECTS OF DIETARY LUPIN MEAL INCLUSION IN SNAPPER AND COBIA

The high survival rates in snapper and cobia fed high dietary LKM is a desirable output for the development of sustainable feeding practices for these species. However, past studies have indicated higher mortality rates in cobia (Lunger *et al.*, 2007; Salze *et al.*, 2010) and snapper (Quartararo *et al.*, 1998a) when they were fed high dietary levels of alternative protein sources.

In the present study, the growth performances decreased as the dietary LKM levels increased in both snapper ($Y = 1.813 – 0.0122X$; $R^2 = 0.986$ and $P < 0.001$, Figure 5.5.1) and cobia ($Y = 3.484 – 0.017X$; $R^2 = 0.887$ and $P < 0.05$, Figure 5.5.1). This pattern is in agreement with previous studies in rainbow trout fed diets containing yellow lupin (Glencross *et al.*, 2004b) or dehulled narrow-leafed lupin (Farhangi & Carter, 2001), or in gilthead sea bream fed narrow-leafed lupin seed (Robaina *et al.*, 1995). The results of our study showed that up to 210 g/kg LKM can be included in cobia diet without any compromise in the SGR. Similar results were also reported in rainbow trout fed narrow-leafed lupin kernel meal (Farhangi & Carter, 2001) or yellow lupin kernel meal inclusions (Glencross *et al.*, 2004b). Although, these studies showed that up to 40 % of fishmeal protein could be replaced by lupin meals without any significant changes in fish growth, the fishmeal content of the control diets in these studies (600 g/kg diet) was much higher than that of the current study (320 g/kg diet). In contrast, snapper showed negative growth rates when fed any inclusion level of lupin meal. In addition, the correlation of SGR with inclusion levels of lupin was significantly stronger for snapper than cobia (Fig.1), indicating the stronger influence of dietary lupin kernel meal on growth rate in snapper than cobia.

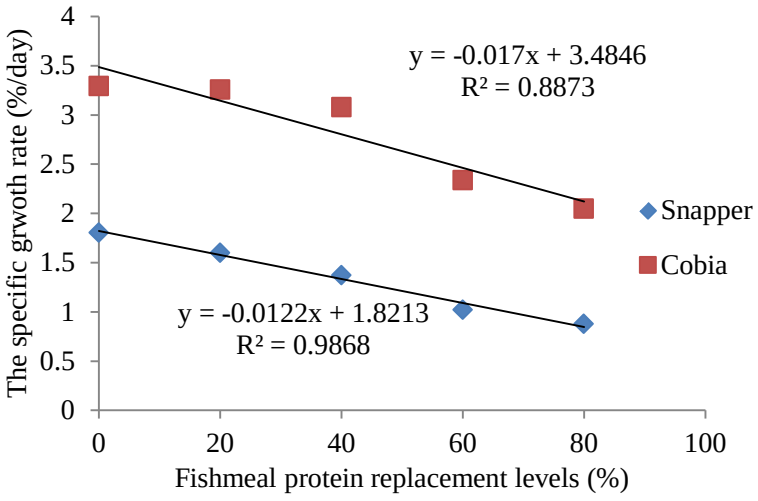


Figure 5.4.1 Linear regression between the SGR and fishmeal protein replacement levels of snapper and cobia

Lysine, methionine and taurine are limiting amino acids in lupin products (Gatlin *et al.*, 2007). The low growth rates of cobia (Zhou *et al.*, 2007; Zhou *et al.*, 2006) and red sea bream (Forster & Ogata, 1998; Matsunari *et al.*, 2008) have been attributed to low availabilities of these amino acids in the diets. In the current study, dietary methionine,

lysine and taurine concentrations got reduced progressively, corresponding with the increase in LKM inclusion levels. Although, the requirements of these amino acids in snapper are still unknown, the lower dietary lysine and taurine in the test diets relative to those required for red sea bream (3.6 % and 0.52 % of the diet, respectively) (Forster & Ogata, 1998; Matsunari *et al.*, 2008) suggest that the deficiency of these amino acids in diets containing LKM may be responsible for the lower growth rates observed in snapper. Relative to snapper, the dietary requirement of lysine, methionine and arginine in juvenile cobia is 2.33, 1.19 and 2.85 %, respectively (Ren *et al.*, 2014; Zhou *et al.*, 2007; Zhou *et al.*, 2006). Although, lupin inclusion diets have lower dietary lysine and methionine levels than fishmeal-based diets, higher dietary lupin inclusion levels are still able to meet the threshold requirements of lysine and methionine in cobia. In the current study, the dietary lysine to arginine ratio was reduced from 1.11 to 0.70, corresponding with the increased LKM. This change may be one of the reasons for the reduced growth rates of cobia in the LKM315 and LKM420 treatments, as reported in cobia (Nguyen *et al.*, 2014). Another reason for the reduced growth rates and feed efficiency in this study is the taurine deficiency in the diets including high levels of lupin, as reported in cobia (Lunger *et al.*, 2007) and rainbow trout (Gaylord *et al.*, 2006) fed dietary plant protein meals. Although taurine can be efficiently synthesised by herbivorous/omnivorous species from other amino acids (Gaylord *et al.*, 2006), the *de novo* synthesis of taurine from cysteine may be poor or not possible in species such as carnivorous fish due to the activity of L-cysteinesulphinate decarboxylase (Lunger *et al.*, 2007), due to their feeding habits (Gaylord *et al.*, 2006).

Similar to the growth rate, the FI of snapper decreased in lupin-based diets; in contrast, the FI of cobia only changed when the fish were fed more than 210 g/kg LKM (Table 5.5.1). This result is inconsistent with studies in barramundi (Glencross *et al.*, 2011) and black sea bream (Zhang *et al.*, 2012), where FI was significantly higher in fish fed lupin meal than in fish fed the control diet, but is consistent with observations in cobia fed plant-based diets (Luo *et al.*, 2012; Romarheim *et al.*, 2008; Zhou *et al.*, 2005). In comparison to snapper, the voluntary feed intake (VFI) was twice as high in cobia, indicating greater feeding capacity of cobia than snapper. Due to the lack of an air-bladder, a faster growing fish as cobia could require greater feed consumption to meet their energy requirement for metabolism, as described in different trouts (Luísa Maria Pinheiro Valente *et al.*, 1998). Sun *et al.* (2006) suggested that the inefficiency in converting energy intake into growth in cobia is due to their relatively higher growth rate, which leads to high food consumption as seen in the present study. Therefore, the

overall FCR was similar for snapper and cobia fed diets containing 0 to 105 g/kg LKM, whereas, the FCR increased in both fish fed diets with higher inclusion levels of lupin (Figure 5.5.2). The finding is also supported by the results in rainbow trout (Farhangi & Carter, 2001; Glencross *et al.*, 2004b) and black seabream (Zhang *et al.*, 2012) fed low levels of lupin meal. The significantly lower FI and increased FCR in snapper and cobia fed high levels of LKM are probably due to the low digestibility of nitrogen, and energy and palatability problems, as reported in cobia (Luo *et al.*, 2012; Luo *et al.*, 2013; Romarheim *et al.*, 2008; Zhou *et al.*, 2005) and snapper (Booth *et al.*, 2012; Quartararo *et al.*, 1998a) fed high plant-protein meals.

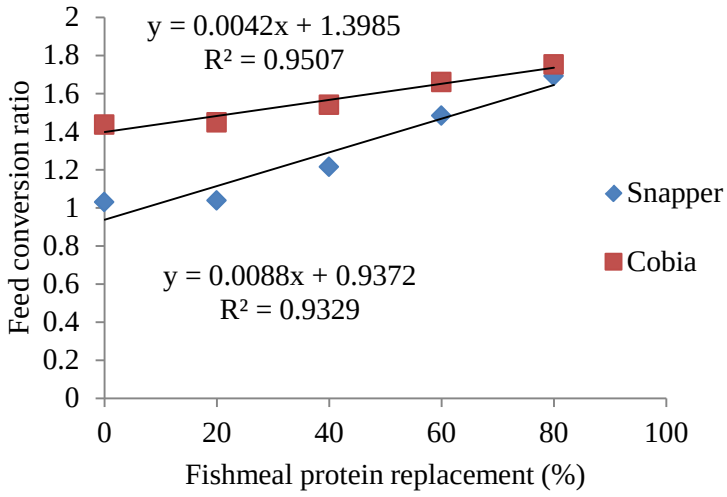


Figure 5.4.2 Linear regression between the FCR and fishmeal protein replacement levels of snapper and cobia

In addition to a decline in growth, an increase in lupin inclusion levels resulted in a decrease in the ER and PR in snapper (Table 5.5.1), similar to rainbow trout (Glencross *et al.*, 2004b). In cobia, the ER and PR were unaffected by the increased inclusion levels of LKM from 0 to 105 g/kg, but significantly declined at higher inclusion levels. Similar results were also seen in cobia fed rapeseed meal (Luo *et al.*, 2012) and soybean meal (Chou *et al.*, 2004). In contrast, Atlantic salmon (Salini & Adams, 2014) and gilthead seabream (Farhangi & Carter, 2001) showed no effects when fed diets containing lupin meal inclusions. The decrease in PR could be related to the decrease in growth and feed intake in both species. Furthermore, the essential amino acid imbalance and the presence of anti-nutrients in diets containing high inclusion levels of LKM could impair the digestibility of the protein (Francis *et al.*, 2001), resulting in a decline in PR in these species, as already reported in cobia (Luo *et al.*, 2012). The lower PER, ER and PR in

cobia than in snapper may be due to the low protein and energy contents in cobia and species-dependent metabolism and growth rates. In marine fish, a large proportion of energy intake is used for metabolism (Sun *et al.*, 2006). Cobia has faster growth rate than snapper; thus it expends higher levels of protein and energy, resulting in lower ER and PR than seen in snapper.

The dry matter digestibility coefficients of the lupin meal were relatively low in both snapper (61.34 – 68.19 %) and cobia (49.06 – 61.34 %). Zhou *et al.* (2004) and Luo *et al.* (2012) also indicated a poor dry matter digestibility of the rapeseed meal and corn gluten meal in cobia, which ranged from 48 - 58.52 % to 84.58 %, respectively. The energy digestibility of lupin meal in cobia in this study was relatively lower than in the published reports in cobia by Zhou *et al.* (2004) and Luo *et al.* (2012), where the energy digestibility of rapeseed meal and corn gluten meal ranged from 83.07 % to 94.23 %, respectively. The relatively low energy digestibility of lupin meal in snapper was similar to that described by Glencross *et al.* (2003b) and Booth *et al.* (2005), who tested different strains of narrow-leafed lupin or soybean meal in snapper. Lupin kernel meal contains high levels of carbohydrates (Gatlin *et al.*, 2007), which is poorly digested by cobia as an energy source (Ren *et al.*, 2011), resulting in low energy digestibility of lupin in this species.

Table 5.4.1 Linear regression between fishmeal replacement levels and tested parameters in snapper and cobia

Indicator	Snapper			Cobia		
	Equation	R^2	P-value	Equation	R^2	P-value
FBW	y = -0.456x + 74.66	0.986	< 0.001	y = -0.7937x + 121.94	0.903	0.013
FI	y = -0.306x + 53.97	0.955	0.004	y = -0.9688x + 148.92	0.866	0.022
PER	y = -0.011x + 2.18	0.954	0.004	y = -0.004x + 1.53	0.968	0.002
PR	y = -0.202x + 38.76	0.952	0.005	y = -0.078x + 24.51	0.869	0.021
ER	y = -0.272x + 44.76	0.965	0.003	y = -0.054x + 22.37	0.922	0.009
ADC-P	y = -0.067x + 93.02	0.922	0.009	y = -0.107x + 89.87	0.985	< 0.001
ADC-E	y = -0.166x + 81.97	0.981	0.001	y = -0.151x + 74.54	0.999	< 0.001
RBC	y = -0.011x + 3.01	0.872	0.020	y = -0.014x + 4.42	0.875	0.019
Hb	y = -0.012x + 6.48	0.947	0.005	y = -0.020x + 7.37	0.877	0.019

FBW final body weight; FI feed intake; PER protein efficiency ratio; PR protein retention; ER energy retention; ADC-P apparent digestibility coefficient of protein; ADC-E apparent digestibility coefficient of energy; RBS red blood cell; Hb haemoglobin.

The protein synthesis and degradation are strongly associated with balanced amino acids (Langar *et al.*, 1993). In this study, fishmeal was replaced by lupin kernel meal without EAAs supplementation, resulting in imbalanced amino acids in the test diets. The significantly reduced protein content in muscles of snapper and cobia fed high inclusion levels of LKM might be due to an imbalance of amino acid in these diets impacting the protein metabolism. The muscle and whole body protein contents were relatively lower in cobia than snapper due to the differences between protein synthesis and degradation in these species. In fish, the synthesised protein proportion used for the growth increases with an increase in the growth rate, as quantified in Atlantic cod (Houlihan *et al.*, 1988). As cobia has a relatively faster growth rate than snapper, more synthesised protein is used to meet its growth potential, resulting in lower protein depositions in cobia. In the current study, the reduced lipid concentrations in the whole-body and muscles were observed in cobia fed diets including more than 210 g/kg LKM, similar to results reported for cobia when fed soybean meal (Zhou *et al.*, 2005), rapeseed meal and corn gluten meal (Luo *et al.*, 2012); Luo *et al.* (2013). This change can be attributed to the low energy intake caused by a low feed intake and energy digestibility. Another reason for the reduced lipid depositions in tissues of cobia can be the taurine deficiency in diets including high levels of lupin due to the important role of taurine in lipid metabolism and oxidative stress responses (Watson *et al.*, 2013). Taurine has been shown to be conditionally indispensable in carnivorous marine fish (Gaylord *et al.*, 2006; Lunger *et al.*, 2007), and taurine supplementation into plant-based diets prevents the reduction of lipid accumulation in tissues of cobia (Lunger *et al.*, 2007). In contrast, the dietary LKM did not show any effects on the lipid contents of the whole-body and muscles of snapper. This pattern is supported by rainbow trout (Borquez *et al.*, 2011), gilthead sea bream (Pereira & Oliva-Teles, 2004) and black sea bream (Zhang *et al.*, 2012). In fish, lipid accumulation occurs in major sites, such as perivisceral fat, muscles and liver tissues, varying among different fish species (Weil *et al.*, 2013). Faster-growing fish generally have greater lipid accumulation (Nortvedt & Tuene, 1998). These reasons might all contribute to explaining the different lipid deposits in different tissues with relatively greater hepatic fat accumulation in cobia than snapper in this study.

The CV of the snapper fed dietary LKM was higher than in the fish fed the control diet because the quantity and quality of food have a strong impact on the size (Fuiman, 2002). In contrast, there was no change in the CV of cobia fed the tested diets. A high CV can be explained as a difficulty in acclimatising to the rearing conditions (Le Boucher *et al.*, 2011). In this study, both snapper and cobia at the same sizes were

collected for feeding trials after acclimation to experimental systems in 2 weeks and feeding available commercial diets to minimise size distributions. Thus, the change in CV of snapper at the end of feeding trial could be attributable to the difficulty in adapting to the test diets. The significantly higher CV of snapper than that of cobia at each inclusion level of lupin also indicated a greater adaptability to dietary inclusion of LKM in cobia than in snapper.

The different changes in haematological characteristics can be useful indicator in evaluating the physiological condition and health of any fish (Kader *et al.*, 2010). Zhou *et al.* (2005) and Sharma *et al.* (2014) demonstrated the relationship between high values of Hb and RBC and good health because Hb and RBC play important roles in oxygen and carbon dioxide transportations in the blood and haemoglobin synthesis (Olugbemi *et al.*, 2010). In this study, the Hb and RBC of cobia were significantly reduced when fed dietary inclusion levels of LKM in excess of 210 g/kg, while this threshold level in snapper was only 105 g/kg LKM (Table 5.5.1). This pattern is consistent with results from cobia (Zhou *et al.*, 2005) and coho salmon *Oncorhynchus kisutchi* (Twibell *et al.*, 2012) fed diets containing high levels of plant-protein meals. Thus, the reduced Hb and RBC concentrations might be a consequence of significantly lower growth performances in snapper and cobia when fed to high dietary LKM in this study.

The intestinal morphology can reflect the physiological adaptation of fish fed different diets (Omnes *et al.*, 2015). In our study, snapper and cobia fed diets including LKM did not show any alterations in the distal intestines. This is consistent with the results of cobia fed soybean-based diets (Romarheim *et al.*, 2008) and rainbow trout fed yellow lupin (Glencross *et al.*, 2004b) or narrow-leafed lupin (Farhangi & Carter, 2001). However, this result is inconsistent with the increment of fat deposits in digestive tracts of Atlantic salmon fed 200 g/kg lupin meal (Gu *et al.*, 2014) or rainbow trout fed 400 – 500 g/kg inclusion of whole grain white lupin (Borquez *et al.*, 2011), probably due to the deficiency of phosphatidylcholine, a major component of the polar lipoprotein surface which plays important role in lipid exporting from the intestinal mucosa to the circulatory system (Gu *et al.*, 2014). The excessive lipid droplet accumulation in the livers of snapper fed high dietary LKM was similar to that reported in mangrove red snapper *Lutjanus argentimaculatus* (Catacutan & Pagador, 2004), Atlantic salmon (Gu *et al.*, 2014) and gilthead seabream (Robaina *et al.*, 1995) fed dietary plant meals due to an increase in lipolysis from adipose tissue, as explained for Atlantic salmon (Gu *et al.*, 2014) and gilthead seabream (Bouraoui *et al.*, 2011). Conversely, Glencross *et al.*

(2004b) observed a decrease in lipid droplets in the liver tissues of rainbow trout fed a 50 % yellow lupin inclusion, which could be associated with the reduced energy retention of the diet. In contrast to snapper, cobia fed a LKM420 diet displayed atrophic hepatocytes. Hepatic atrophy in fish caused by mineral toxicity have been shown in yellowtail kingfish (Le & Fotedar, 2014b). However, the reason for the symptom induced by dietary LKM in cobia is not clear and needs further study. Although toxic effects of anti-nutrient factors such as lupinine, sparteine and gramine in lupin products have been demonstrated in animals (Flores-Soto *et al.*, 2006; Olver & Jonker, 1997), the increasing dietary inclusion levels of lupinine or spartein up to 5000 mg/kg did not cause any histopathological alterations in the intestine, liver and kidney of rainbow trout (Serrano *et al.*, 2012; Serrano *et al.*, 2011). In addition, selective breeding has reduced the alkaloids concentration in lupins to insignificant levels (less than 20 mg/kg), and the meal could be readily used by livestock (Duke, 1981). Further, the concentrations of phytic acid, tannin and trypsin inhibitor presented in narrow-leafed lupin kernel meal at the level of 0.50 %, 0.17 % and 0.12 mg/g (Petterson *et al.*, 1997; Pham *et al.*, 2016; Vo *et al.*, 2015) are relatively lower compared to soybean meal and rapeseed meal (Glencross, 2001; Luo *et al.*, 2012), indicating that the ANFs in the diet including 210 g/kg LKM is unlikely to cause any histopathological changes in the digestive tissues of either snapper or cobia.

5.5. ROLES OF MINERAL SUPPLEMENTATION IN FISH FED PLANT-BASED DIETS

Compared with plant-derived ingredients, fishmeal has been shown to be excellent source of not only protein, essential amino acids, but also macro-nutrients and micro-nutrients in fish diets (Hertrampf & Piedad-Pascual, 2000). Therefore, the substitution of fishmeal with plant protein sources in aqua-feeds can change the nutritional composition, altering the uptake and digestion of minerals, consequently changing the nutritional requirements of the fish (Antony Jesu Prabhu *et al.*, 2016; Barrows *et al.*, 2010; Read *et al.*, 2014). Besides, the anti-nutritional factors, such as phytic acid and tannin presented in plant-derived ingredients have been reported to be involved the reduction of mineral bioavailability in fish (Antony Jesu Prabhu *et al.*, 2016) due to their disruptions in digestion and metabolism of minerals (Antony Jesu Prabhu *et al.*, 2016). Thus, fish fed high inclusion levels of plant feedstuffs may require mineral supplementation to diminish the negative impacts of ANFs, meanwhile improve the growth and feed utilisation.

Barrows *et al.* (2008) demonstrated that rainbow trout fed plant-based diet required premix vitamin supplementation to improve growth and feed utilisation at a higher concentration than those previously determined for purified diet. Similarly, the dietary minerals, such as zinc and copper supplementation were essential for the trout fed plant-based diets (Barrows *et al.*, 2010; Read *et al.*, 2014; Welker *et al.*, 2016). The authors also reported that quantitative mineral requirements recommend by the NRC (1993) and NRC (2011) may result in the nutrient deficiency in the trout fed plant-derived diets.

Although the fishmeal-based diets have been reported to contain adequate Se concentration to meet the nutritional requirement in fish, however, the increase in its price, the high demand for animal feeds and limiting of its availability have led to efforts to reduce the fishmeal reliance by increasing of cost-effective alternative protein ingredients in marine fish diets (Olsen & Hasan, 2012; Welker *et al.*, 2016). The percentage of fishmeal in marine fish diet rapidly dropped from 50% in 1995 to only 26 % in 2010 and had been predicted to continuously reduce to 12 % in 2020 (Olsen & Hasan, 2012). Besides, due to the significantly lower Se digestibility from fishmeal (38.48 – 47 %) than from SeMet or Se-yeast (89.48 – 92 %) (Bell & Cowey, 1989; Le & Fotedar, 2014a; Watanabe *et al.*, 1997), the relatively lower Se concentration in plant-derived ingredients compared to fishmeal (Antony Jesu Prabhu *et al.*, 2016; Welker *et al.*, 2016) as well the interaction between Se and other minerals such as copper, sulphur, mercury (Watanabe et al., 1997) and vitamin E (Le *et al.*, 2014b; Lin & Shiau, 2009), thus, fish fed high inclusion levels of plant-derived ingredients may require dietary Se fortification to meet their nutritional requirement (Abdel-Tawwab *et al.*, 2007; Ilham *et al.*, 2016a; Le & Fotedar, 2013; Zhu *et al.*, 2012).

Recently, the beneficial effects of Se supplementation have been also demonstrated in fish fed plant-based diets (Abdel-Tawwab *et al.*, 2007; Ilham *et al.*, 2016a; Ilham *et al.*, 2016b), in which African catfish and barramundi showed improved growth and feed utilisation efficiency after being fed plant-based diets supplemented with organic Se. Besides, barramundi fed Se-supplemental plant-based diets also demonstrated the improvement in histopathological performances (Ilham *et al.*, 2016a). The reason for this can be due to the increased quantity and activities of digestive enzymes (Chaudhary *et al.*, 2010) as well as the enhancement in health status of fish (Abdel-Tawwab *et al.*, 2007; Le *et al.*, 2014a) stimulated by dietary Se supplementation.

5.6. EFFECTS OF DIETARY ORGANIC SELENIUM SUPPLEMENTATION IN COBIA FED LUPIN-BASED DIETS

In the absence of Se supplementation, cobia fed high dietary levels of LKM showed reduced FBW, SGR, FI and nutrient digestibility, corresponding to increased FCR. This was consistent when cobia were fed soybean meal (Chou *et al.*, 2004; Zhou *et al.*, 2005) and rapeseed meal (Luo *et al.*, 2012). The reduced growth rates in cobia fed 315 g/kg LKM can be linked to the depression in FI as FI is directly related to weight gain (Espe *et al.*, 2012). Therefore, it is essential to maintain an equal feed acceptability in order to evaluate the performance of the fishmeal-substituted diets (Espe *et al.*, 2012).

The suppression of FI has been attributed to the deficiency of essential amino acids in lupin-based diets (Jobling *et al.*, 2007), probably due to low concentrations of lysine and methionine in lupin products (Gatlin *et al.*, 2007) as shown by relatively lower growth and feed efficiency in cobia by Zhou *et al.* (2006) and Zhou *et al.* (2007). The dietary requirements for lysine and methionine of juvenile cobia are 2.33 % and 1.05 %, respectively (Zhou *et al.*, 2007; Zhou *et al.*, 2006). Although lupin inclusion diets contain lower dietary lysine and methionine concentrations than fishmeal-based diets, however, the higher dietary lupin inclusion levels in the current study are still able to meet the threshold requirement of lysine and methionine for juvenile cobia.

The high inclusion levels of dietary plant-derived ingredients in aqua-feeds also result in an increased proportion of dietary anti-nutrients (Francis *et al.*, 2001; Gatlin *et al.*, 2007). The interactions between anti-nutritional factors presented in protein ingredients and micronutrients can also impact the absorption and metabolism of these micronutrients (Read *et al.*, 2014). The molecules in phytate can strongly attach to minerals such as calcium, zinc, copper, iron, manganese, nickel and Se to form insoluble compounds, reducing the bioavailability of these minerals (Francis *et al.*, 2001). The chelation between phytate and cation groups in protein, amino acids and lipid can also result in a reduction in the digestibility of these nutrients (Francis *et al.*, 2001). Meanwhile, the metabolism of minerals in fish might also be compromised due to the presence of other anti-nutritional factors, such as tannin, saponin, glucosinolates and gossypol (Antony Jesu Prabhu *et al.*, 2016), consequently depressing the growth, feed utilisation and mineral absorption in fish, as observed in Atlantic salmon (Storebakken *et al.*, 1998) and channel catfish (Satoh *et al.*, 1989). However, there could be beneficial effects of phytate at low concentrations on growth and feed intake when fed to blue

tilapia *Oreochromis aureus* (McClain & Gatlin, 1988) and Atlantic salmon (Carter & Hauler, 2000). Relative to other plant ingredients, narrow-leafed LKM contains insignificant levels of phytic acid (0.53 %) and tannins (0.16 %), resulting in low dietary phytic acid and tannin concentrations in the test diets. In addition, the saponins, trypsin and chymotrypsin inhibitors found in the narrow-leafed LKM at low concentrations (574, 0.12 and 0.6 mg/kg, respectively) are unlikely to interfere with the uptake and absorption of nutrients in fish (Petterson, 2000).

One possible reason for the reduced nutrient digestibility in cobia fed lupin-based diets could be the presence of oligosaccharides in the diets. The narrow-leafed LKM contains relatively high levels of oligosaccharides in the forms of raffinose, stachyose and verbascose (Glencross, 2001; Petterson, 2000), which interact with the digestion of other nutrients (van Barneveld, 1999) and prevent the activity of digestive enzymes and substrate transportation in the intestine (Francis *et al.*, 2001), thus reducing nutrient digestibility as seen in rainbow trout (Glencross *et al.*, 2003a) and Atlantic salmon (Refstie *et al.*, 1998). Lupin meal also contains a significant amount of carbohydrates (Gatlin *et al.*, 2007), which are poorly digested by cobia as an energy source (Ren *et al.*, 2011), resulting in low energy digestibility. In contrast, cobia fed dietary LKM supplemented with organic Se showed improved growth, feed efficiency and nutrient digestibility compared with the fish fed diets lacking Se supplementation. A positive relationship between nutrient digestibility and dietary Se supplementation has been demonstrated in pigs (Chaudhary *et al.*, 2010) and sheep (Shi *et al.*, 2011), due to the increased quantity and activities of digestive enzymes induced by dietary Se (Chaudhary *et al.*, 2010). However, the effects of interaction between dietary Se and plant-derived protein on feed efficiency and nutrient digestibility has been reported in only one study, where barramundi showed increased protein digestibility after being fed Se-supplemented lupin-based diets (Ilham *et al.*, 2016a). In the case of soybean, African catfish fed plant-based diets with supplemented Se demonstrated increased activities of protein metabolism enzymes and FI (Abdel-Tawwab *et al.*, 2007). This could be indirectly associated with the complex mechanism of amino acid-chelated Se (AAC-Se) ingestion, resulting in increased essential trace element sources when high AAC-Se is absorbed into mucosal tissues (Ilham *et al.*, 2016a). These elements act as a cofactor in the synthesis of hydrolytic enzymes, such as gastro-intestinal GPx, which plays important roles in defending the intestinal mucosal integrity (Lindh, 2013), stimulating nutrient digestion in fish (Ilham *et al.*, 2016a).

Table 5.6.1 Performances of fish fed plant-derived ingredients supplemented with Se

Species	Plant sources	Se forms	Effects of Se	References
African catfish *Clarias gariepinus*	Soybean	Se-yeast	Improved growth, feed utilisation efficiency. Enhancement in health status and GPx activity.	Abdel-Tawwab *et al.* (2007)
Rainbow trout *Oncorhynchus mykiss*	Soybean	Se-yeast	Increased tissue Se deposition and GPx activity	Fontagné-Dicharry *et al.* (2015)
Barramundi *Lates calcarifer*	Lupin kernel meal	Se-yeast	Increases in growth, protein digestibility, GPx activity and Se depositions	Ilham *et al.* (2016a)
Barramundi *Lates calcarifer*	Soybean	Se-yeast	Improved growth, haematological parameters. Increased GPx activity and tissue Se levels.	Ilham *et al.* (2016b)
Cobia *Rachycentron canadum*	Lupin kernel meal	Se-yeast	Improved nutrient digestibility, growth and feed efficiency. Increased tissue Se accumulations and haematological performances.	Pham *et al.* (2016)

The dietary Se requirement has been established for juvenile cobia using purified diet (Liu *et al.*, 2010), however, the biological effects of dietary Se on the feed utilisation efficiency of plant-derived ingredients, such as LKM, in this species are still unknown. In the current study, though increased levels of dietary LKM resulted in a corresponding decrease in dietary Se levels, the minimum dietary Se was still higher than threshold requirement for cobia, using a casein-based diet (Liu *et al.*, 2010). However, the improved growth and feed utilisation efficiency in cobia fed supplemental Se lupin-based diets indicated that the endogenous dietary Se in the lupin-based diets may not meet the nutritional requirement for juvenile cobia. Feed ingredients generally contain a varied amount of Se, with a higher availability of Se from plant-derived ingredients than from fishmeal (Watanabe *et al.*, 1997). To determine the Se requirement in fish while overcoming the complications of the varied Se availabilities in feed ingredients, casein is generally used as the sole protein source in purified or semi-purified diets (Hilton *et al.*, 1980; Lin, 2014; Lin & Shiau, 2005; Liu *et al.*, 2010). However, the use of casein, compared with fishmeal or krill meal, in commercial diets becomes challenging due to its high price and poor feed intake and growth rates (Hertrampf & Piedad-Pascual, 2000). Further, in the current study, juvenile cobia fed diets containing various protein ingredients resulted in considerably higher SGR than reported by Liu *et al.* (2010) in the fish fed a casein-based diet. The higher metabolic rates associated with faster-growing

fish require sufficient energy to maximize their growth potential (DeVries & Eastman, 1981), resulting in a need to take up more nutrients, including Se. The optimum dietary Se concentrations quantified for most fish species fed casein-based diets ranged from 0.15 to 0.98 mg/kg (Hilton *et al.*, 1980; Lin, 2014; Lin & Shiau, 2005; Liu *et al.*, 2010), whereas fish fed fishmeal or plant meal-based diets resulted in relatively higher dietary Se requirement levels, ranging from 1.62 to 5.35 mg/kg (Abdel-Tawwab *et al.*, 2007; Le & Fotedar, 2013; Zhu *et al.*, 2012). Even though, fishmeal-based diets can provide adequate Se to meet the nutritional requirements of some species (Watanabe *et al.*, 1997), the significantly lower Se digestibility from fishmeal (38.48 – 47 %) compared with SeMet or Se-yeast (89.48 – 92 %) (Le & Fotedar, 2014a; Watanabe *et al.*, 1997) and relatively lower concentration of Se in lupin kernel meal (0.35 mg/kg) than fishmeal (4.37 mg/kg) may result in Se deficiency when fishmeal protein is replaced with lupin kernel protein, as seen in barramundi (Ilham *et al.*, 2016a) and cobia as in the current study. Moreover, Barrows *et al.* (2010) and Read *et al.* (2014) also reported improved weight gain in rainbow trout fed plant-based diets supplemented with dietary macro-minerals and inositol and/or dietary copper and zinc at higher levels than those previously quantified by the NRC (1993) for the species. Similarly, the beneficial effects of Se fortification were also observed in African catfish fed soybean-based diets (Abdel-Tawwab *et al.*, 2007) and barramundi fed plant-based diets (Ilham *et al.*, 2016a), which may be, in part, a reason for the enhancement of growth and feed utilisation performances in the current study (Table 5.7.1).

The values of RBC and Hb can provide useful information about oxidative stress and toxicological impacts in fish as RBCs membranes may be damaged by free radicals produced in erythrocytes, thus, changing the integrity, size and quantity of RBC, and finally altering fish health (Kiron *et al.*, 2004). In this study, reduced Hb and RBCs concentrations was shown in cobia fed dietary lupin meal without Se supplementation, consistent with a previous report in cobia (Zhou *et al.*, 2005) fed soybean-based diets. The restriction on RBCs production and Hb synthesis imposed by adverse effects of anti-nutrients, as previously reported in cobia (Zhou *et al.*, 2005), could constitute the reason. Conversely, cobia fed dietary lupin meal supplemented with Se did not show any significant differences in haematological performance compared with fish fed the control diet. Increased RBC and Hb have been linked to improved health status in fish (Abdel-Tawwab *et al.*, 2007), due to their important roles in oxygen and carbon dioxide transportation in the blood and haemoglobin synthesis (Olugbemi *et al.*, 2010). The increases in the RBC and Hb values in the cobia fed lupin-based diets supplemented with

Se can be attributed to the enhancement of fish health stimulated by Se supplementation, as described in other fish species (Abdel-Tawwab *et al.*, 2007; Ilham *et al.*, 2016a).

In the current study, dietary LKM did not have any effects on protein, ash and dry matter levels in the muscles of cobia, irrespective of Se supplementation. This is consistent with the study in gilthead sea bream (Pereira & Oliva-Teles, 2004) and black sea bream (Zhang *et al.*, 2012) when fed lupin inclusion diets. A deficiency of taurine in plant-based diets has been linked to the reduced lipid deposition in the tissues of cobia (Lunger *et al.*, 2007). However, in this study, taurine was added at 5 g/kg to satisfy the nutritional needs of cobia as quantified by Watson *et al.* (2014a). Thus, the reduced muscle lipid contents of cobia fed 315 g/kg LKM diet in the current study might be due to the low energy intake and energy digestibility, similar to those reported in cobia fed soybean meal (Zhou *et al.*, 2005), rapeseed meal or corn gluten meal (Luo *et al.*, 2012; Luo *et al.*, 2013). The Se depositions in cobia tissues had a strong linear relationship with dietary Se levels, which can be used as a biomarker of dietary Se delineation, as reported in other carnivorous marine fish (Ilham *et al.*, 2016a; Le & Fotedar, 2014b).

In this study, cobia showed less susceptible to dietary LKM than snapper, where up to 210 mg/kg LKM could be incorporated in diet without compromising growth and feed intake of cobia. However, the increased FCR and reduced RBC and Hb also were observed in cobia fed this diet. Besides, the results in this study indicated that cobia, but not snapper fed a commercial diet containing different protein sources required Se supplementation to improve their potential growth and feed utilisation efficiency at a higher concentration than previously determined for purified diet. Thus, it was proposed that dietary Se supplementation could improve the beneficial effects of lupin meal inclusion in cobia, but not for snapper due to their relatively low Se requirement.

CHAPTER 6. CONCLUSIONS, RESEARCH LIMITATION AND RECOMMENDATIONS

6.1 CONCLUSIONS

The following points display major conclusions based on the results achieved from the current study:

- Australian snapper requires a lower concentration of dietary protein than the currently used diets for barramundi diet.
- Se requirements of Australian snapper are low and can be met by endogenous Se in the feed ingredients.
- The dietary Se levels of 1.76 and 2.77 mg/kg in the commercial and basal diets, respectively, can be growth retardant and toxic to the Australian snapper.
- Cobia fed a commercially available feed containing 1.15 mg/kg Se shows signs of Se deficiency, resulting in reduced growth, feed intake and GPx activities.
- Dietary Se of 3.14 mg/kg has negative effects on the physiological and histological performances of juvenile cobia.
- The optimal dietary Se level required for juvenile cobia fed a commercial diet is 2.32 mg/kg based on maximal specific growth rate (SGR) using quadratic regression analysis.
- Cobia shows less susceptibility to dietary lupin kernel meal than snapper. The snapper responds negatively to any levels of LKM inclusion, while the dietary inclusion level of 105 g/kg LKM do not impair the growth rates and feed efficiency, digestibility and health of cobia.
- Up to 210 g/kg LKM can be incorporated in cobia diet without compromising growth and feed intake.
- Cobia and snapper are capable to digest protein from LKM. Cobia fed diets including more than 210 g/kg LKM results in the reduced nutrient depositions and distinct haematological characteristics, whereas this level is only 105 g/kg in snapper.
- Dietary LKM of 420 g/kg results in the histopathological lesions in the livers of both snapper and cobia.
- Dietary Se supplementation in lupin-based diet enhances growth and feed utilisation of juvenile cobia. Up to 40 % of fishmeal protein replaced with

narrow-leafed lupin kernel meal protein with Se supplementation do not cause any effects on the FW, SGR, FI and FCR of cobia.

- The dietary Se supplementation has no beneficial effects on the muscle composition and amino acid profiles of cobia, but significantly improves the haematological parameters and nutrient digestibility of cobia.

6.2 LIMITATIONS

Though the major objectives have been achieved in this study, some limitations are unavoidable. First, due to the lack of small juvenile snapper, the first feeding trial was conducted with snapper at larger size than anticipated. As the bigger fish may be less sensitive to dietary Se than the small one, thus dietary Se supplementation may have different effects in juvenile snapper. Though, the LKM used in this study contains relatively lower phytic acid and tannins concentrations, which is unlikely to impair nutritional and physiological performances of snapper and cobia fed lupin-based diets; however, the levels of other ANFs such as oligosaccharides and alkaloids have not determined in this study. Third, based on the results attained in the first experiment, it was proposed that the endogenous Se presented in various protein ingredients in the basal diet or commercial marine fish diets could provide sufficient Se to meet nutritional requirement in snapper. Thus, no experiment was carried out to evaluate the effects of dietary Se supplementation in snapper fed various inclusion levels of lupin kernel meal. However, the findings in cobia indicated that there was significant interaction between dietary Se and lupin on the growth and feed utilisation. Though, the dietary Se requirement is low in snapper, there is a possibility that the interaction of Se and plant-derived proteins may enhance the growth and physiological performances when snapper are fed to a plant-based diet.

6.3 RECOMMENDATIONS

Based on the results and the limitations from this research, the following recommendations are made for future research.

- The effects of dietary Se supplementation should be evaluated in smaller sized snapper.
- As the toxicity of Se may be dependent on fish size and feed formulations, the future research needs to be conducted on different life stages of both snapper and cobia to investigate the toxicity of Se.

- The interactive effects of organic Se supplementation and plant based diet on the physiology of snapper.
- The results in this study should be carried out in a commercial fish farming environment to validate these results.

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