AN INVESTIGATION INTO THE EFFECT OF DIETARY PROTEIN AND FISHMEAL REPLACEMENT IN JUVENILE YELLOWTAIL, *SERIOLA LALANDI* (PISCES: CARANGIDAE) IN A RECIRCULATING AQUACULTURE SYSTEM

Submitted in fulfillment of the requirements for the degree of

Master of Science in Zoology

By

Apelele Manjingolo

(201515815)

Department of Zoology and Entomology

Faculty of Science and Agriculture

University of Fort Hare

Alice

Supervisor: Prof NG Vine

January 2023

Table of contents

Declaration ii

Acknowledgements iii

ABSTRACT iv

CHAPTER 1 1

GENERAL INTRODUCTION 1

Research Aim 10

Objectives 10

CHAPTER 2 12

GENERAL MATERIALS AND METHODS 12

CHAPTER 3 20

THE EFFECT OF DIETARY PROTEIN LEVEL ON SURVIVAL, GROWTH, AND FOOD CONVERSION RATIO OF JUVENILE YELLOWTAIL IN A RECIRCULATING AQUACULTURE SYSTEM (RAS) 20

Introduction 20

Materials and methods 22

Results 26

Discussion 32

CHAPTER 4 37

THE EFFECT OF REPLACING DIETARY FISHMEAL ON THE SURVIVAL, GROWTH, FCR, AND HEALTH OF JUVENILE YELLOWTAIL, *SERIOLA LALANDI* IN A RECIRCULATING AQUACULTURE SYSTEM (RAS). 38

Introduction 38

Materials and methods 40

Results 44

Discussion 54

CHAPTER 5 58

GENERAL DISCUSSION 58

REFERENCES 61

Declaration

I, Apelele Manjingolo, student number (**201515815**), hereby certify that this dissertation is my original work and has not been submitted in this or any other form to any University other than the University of Fort Hare, Alice, South Africa. Where use has been made of the research of others, it has been duly acknowledged in this text.

Signed:  Date: 31/01/2023

I, Apelele Manjingolo, student number (**201515815**) hereby declare that I am fully aware of the University of Fort Hare's policy on plagiarism and I have taken every precaution to comply with the regulations.

Signed: 

I, Apelele Manjingolo, student number (**201515815**) hereby declare that I am fully aware of the University of Fort Hare's policy on research ethics and I have taken every precaution to comply with the regulations. I have obtained an ethical clearance certificate from the University of Fort Hare's Research Ethics Committee and my reference number is the following: **VIN012SMAN01/20/A**

Signed: 

Acknowledgements

I would like to dedicate this thesis to my late mother, Nozamile Manjingolo brother, Musa Manjingolo and best friend, Soso Somacala.

Firstly, I want to thank my supervisor, Prof Niall Vine for the support, guidance and encouragement throughout the process. You left a permanent mark in my life, and I will be forever grateful.

I would like to thank the National Research Foundation and ACEP Phuhlisa Programme at the South African Institute for Biodiversity for funding my project.

Much appreciation to Kingfish Enterprises (Pty) Ltd for donating fish to the project and for space to conduct my experiments and write up. To, Prof Cliff Jones from Rhodes University and Dirk Weich from Marifeed (Pty) Ltd- thank you for assisting with formulation and manufacturing of the experiment feeds. Appreciation to the Soil Fertility and Analytical Services at KwaZulu Department of Agriculture and Rural Development for analyzing my feed and fish samples and African Aquatic Vet Services for the preparation of my histology slides and Dr Gilliam Taylor for assisting with their interpretation.

I would also like to thank the staff at Kingfish Enterprises (Pty) Ltd for always assisting whenever I needed help. To my colleagues, Dr Aldi Nel, Ziyanda Mzozo, Masi Bulube, Bongi Mngqushu and friends, Salizwa Malindi, Aphile Matam, Lubabalo and Luthando Mncanca thank you for the constant support and assistance with the experiments, much appreciated.

Special thanks to my family for always supporting and believing in me, it means a lot.

ABSTRACT

The yellowtail kingfish, *Seriola lalandi*, is a carnivorous species, which require high levels (>40%) of protein in its diet of which a significant portion comes from fishmeal. Fishmeal is usually the main ingredient when formulating a diet for carnivorous fish because of its high protein content, good digestibility and balanced essential amino acid profile. The use of fishmeal in diet formulations has decreased because of high prices and short supply. Aquaculturists have little alternative but to reduce the amount used or replace it with alternative suitable protein sources. This study aimed to contribute to the optimization of protein requirements and fishmeal replacement in the diet of juvenile yellowtail in a Recirculating Aquaculture System (RAS). This study was conducted at the marine finfish farm, Kingfish Enterprises (Pty) Ltd in the East London Industrial Development Zone (ELIDZ) in a purpose designed RAS.

The first experiment investigated the effect of dietary protein level on survival, growth, food conversion ratio (FCR) and health of juvenile yellowtail in a RAS. Four iso-calorific diets containing different dietary protein levels (38%, 44%, 50% and 56%) were formulated. Each diet was fed to three RAS tanks holding juvenile yellowtail (30 fish per tank) for 49 days. Dietary protein levels did not influence survival of juvenile yellowtail. Specific growth rate (SGR) ($p=0.003$) was greater in fish fed 56% protein compared to fish fed 38% protein but there were no differences between the 56%, 50% and 44% protein diets. There were no differences in protein efficiency ratio (PER) between the four diets. FCR decreased with increasing protein level with fish being fed the 56% protein yielding the lowest FCR (1.26) compared to fish fed 38% protein (1.76) ($p=0.003$), however, there was no difference between the 44%, 50% and 56% protein levels. The hepatocytes of fish fed high dietary lipid content (diet with 38% and 44% protein) showed large lipid vacuole zones in the cytoplasm ($p<0.001$). Body protein, ash, moisture, and lipid was not affected by different dietary protein levels. In terms of producing a

diet with best growth at the lowest cost, a 44% protein diet is considered as optimal for juvenile yellowtail in RAS.

The second experiment investigated the effect of partial and full replacement of fishmeal with a mixture of alternative protein sources (chicken meal, blood meal, and soybean meal in equal proportions) that are more sustainable and less costly. Four experimental diets were formulated to be iso-nitrogenous (44% protein) and iso-calorific (19.50 Mg/Kg) as follows: (1) a control diet with fishmeal as the only protein source (which was the same formulation as the 44% diet used in the first experiment), (2) control diet in which 33% of fishmeal was replaced with the protein mixture, (3) control diet in which 67% of fishmeal was replaced with the protein mixture and (4) control diet in which 100% of fishmeal was replaced with the protein mixture. Different fishmeal levels in the diets did not have an effect on the survival on juvenile yellowtail. Specific growth rate (SGR) was greater for fish fed 67 and 100% fishmeal ($p= 0.017$) compared to fish fed 0% fishmeal. There were, however, no differences in SGR between fish fed 33, 67, and 100% fishmeal. Condition factor (CF) was significantly affected by the interaction between dietary treatment and time with average values lower at the end of experiment compared to the start of the experiment. On day 28 ($p= 0.02$) and 49 ($p= 0.01$), CF of fish fed 67 and 100% fishmeal diet differed significantly from those fed the 0% fishmeal diet but 33, 67, and 100% fishmeal diets did not differ from each other . Feed conversion ratio (FCR) was significantly affected by fishmeal replacement with fish on fishmeal-containing diets showing lower FCRs compared to those on fishmeal-free diets ($p= 0.001$). Fish fed diets containing 67 and 100% fishmeal had low PER (0.026) compared to fish fed 0% fishmeal. There were, however, no differences in PER between fish fed 33, 67, and 100% fishmeal. Hepatosomatic index (HSI) was not affected by levels of fishmeal replacement in the diet. Body protein, ash, moisture, and lipid was not affected by partial and full replacement of fishmeal. The results indicate that full replacement of fishmeal (0% diet) had the worst SGR,

FCR, and PER. The remaining three diets were statistically similar which suggests that a diet with 33% fishmeal produces as good growth, FCR and PER as 67 and 100% fishmeal diets. However, as there were also similarities in SGR and PER for the 0 and 33% fishmeal diets, it is therefore recommended that future studies focus on the region between 33-67% to find the optimal break point for the various growth indicators.

This study has provided foundation for the formulation of yellowtail kingfish diet which is suitable for use in a RAS.

CHAPTER 1

GENERAL INTRODUCTION

The human population of the world is increasing rapidly and by 2050 it is expected to reach 9 billion (Kobayashi et al. 2013). The increase in world population has resulted in an increased demand for food. This has resulted in rapid increase of agriculture production over the years exceeding the rate of population growth.

Aquaculture in the world

Aquaculture is the fastest growing food production industry in the world. The growing aquaculture industry can play a pivotal role in the meeting food security demands (Kobayashi et al. 2013).World aquaculture production has reached 214 million tons in 2020 with aquatic animals accounting for a total of 178 million tons while algae account for 36 million tons (Sofia 2022).. Yellowtail kingfish, *Seriola lalandi,* is one of the species being cultured in South Africa.

Yellowtail kingfish, *Seriola lalandi,* and a review of its culture

Seriola lalandi belongs to the Carangidae family which has 151 species and 32 genera. *Seriola lalandi* is a fast-swimming migratory species that lives in various habitats that include the open sea, estuaries, reef pinnacles and surf zones (Booth et al. 2010). Yellowtail kingfish can grow up to 60 kg, however those that inhabit South African waters do not exceed 20 kg. Yellowtail kingfish (*S. lalandi*), amberjack (*Seriola dumerili*) and highfin amberjack (*Seriola rivoliana*) are three *Seriola* spp. that are commonly found in warm temperate and tropical waters east of Port Elizabeth in South Africa (Dunn 2014).

Seriola lalandi is an important aquaculture species in Japan, Australia and New Zealand (Kolkovski and Sakakura 2007, Ramírez and Romero 2017). This specie's fast growth, flesh texture, excellent taste and established markets makes it an ideal species for aquaculture (Kolkovski and Sakakura 2007, Buentello et al. 2015, Ramírez and Romero 2017). Under optimal conditions, *Seriola* spp. are able grow up to 4 kg in 15 to 18 months (Kolkovski and Sakakura 2007) and are typically used for sushi and sashimi as they are therefore highly valued in Asian countries including Singapore, Japan, Hongkong and Korea (Bowyer 2012).

Fish nutrition

The intake of essential nutrients is the prerequisite for the successful production and growth of all farmed fish (Irish 1997, Bowyer et al. 2013). Nutrients are described as constituents of diet the that are physiologically important in animal and cellular metabolism (Davis 2015). Nutrients are important for health, reproduction and optimal growth. They are sometimes grouped into macronutrients which includes lipids, protein and carbohydrate and micronutrients which constitutes vitamins and minerals, and cultured fish obtain these nutrients from their feeds (Davis 2015).

Tacon et al. (2013) and Tacon and Metian (2015) proposed the following factors and considerations that must be employed by farmers when selecting feed input for a selected fish species:

- The stocking density and the suitable culture system of that species.
- The availability of commercially prepared feeds of that species.
- The cost and local market availability of feed ingredients of the selected species.
- The market value and feeding habit of the selected species.
- The financial means of the farmer in order to acquire feeds for selected species and culture system.

In a commercial finfish farming, feed is the most expensive component, constituting an average of 42% to 52% of total operational costs of the farm (Irish 1997, Woolley 2009, Trushenski et al. 2011, Craig and Helfrich 2017). The high cost of feed has resulted in research on the diet that produces the best growth at the lowest cost. Formulated diets should therefore have all the essential nutrients that will aid in producing best possible growth and survival of the fish at minimum cost (Woolley 2009). It is also important to consider the characteristics of the feed when formulating diets of fish which are based on their nutritional requirements. These characteristics of the feed include, shape, size, taste and texture as their play a huge role on whether the fish feed on the diet or not (Gordon 1999, Woolley 2009).

The growth of juvenile fish is highly dependent on protein and out of all the nutrients, protein is the most expensive. Therefore careful attention must be given to optimizing the protein content of the diet (Irish 1997, Green 2009, Woolley 2009). Protein requirement is not the same for all species under culture conditions (Irish 1997) (Table 1.1). It is crucial to consider the amino acid profile, digestibility and energy level when determining the optimum dietary protein level. This is important because if one or more of them is out of proper balance will result in overestimation of optimum dietary protein level(Irish 1997).

Protein requirements for carnivorous fish are generally higher than that of omnivorous and herbivorous fish (Bowyer et al. 2013, Craig and Helfrich 2017). Proteins levels in aquafeeds averages 28-32% for catfish, 30-35% for shrimp, 40-45% for trout and other marine finfish (Craig and Helfrich 2017). The rearing environment also has an effect on finfish protein requirements. Protein requirements are generally lower for fish reared in low density culture systems such as ponds compared to fish reared in high density culture systems such as recirculating aquaculture system (RAS) (Craig and Helfrich 2017). The protein requirement of finfish generally decreases as they grow older. (Craig and Helfrich 2017).

Yellowtail, like other carnivorous fish require high protein levels in their diet for optimum growth (Moran et al. 2010, Stuart et al. 2018) (Table 1.1). The high dietary protein levels required by yellowtail is because they use protein for both energy and growth as other carnivorous species (Moran et al. 2010). According to Nakada (2008), cultured yellowtail were previously fed raw frozen fish before formulated diets. Nakada (2008), reported that introduction of extruded pellets improved the feed conversion ratio of yellowtail compared to raw frozen fish. Yellowtail are currently fed extruded pellets with trash fish typically only reserved for broodstock feeding (Nakada 2008, Booth et al. 2010).

Table 1.1: Dietary protein levels of formulated aquaculture diets used in the farming of marine carnivorous finfish species.

Species	% Crude protein level	References
Dicentrarchus labrax	38	El-Dahhar et al. (2006)
Sparus aurata	44	Moutinho et al. (2016)
Sciaenops ocellatus	44	Thomas et al. (1991)
Seriola lalandi	44	This study
Sciaenops ocellatus	44	Daniels and Robinson (1986)
Argyrosomus japonicus	45	Daniel (2004)
Paralichthys olivaceus	45	Li et al. (2021)
Seriola lalandi	48.5	Jirsa et al. (2014)
Salmo trutta caspius	50	Ramezani (2009)
Solea senegalensis	53	Rema et al. (2012)
Limanda. ferruginea	55	Dwyer et al. (2002)
Epinephelus malabaricus	55	Tuan and Williams (2007)
Lutjanus argentiventris	55	Maldonado-García et al. (2012)

Paralichthys olivaceus	56	Seo et al. (2022)
Pseudopleuronectes yokohame	58.8	Cho et al. (2021)

Fishmeal has been use as the main source of protein when formulating a diet for carnivorous fish because of its excellent palatability, balanced amino acid profile, high protein, good digestibility and essential fatty acids content (Bowyer et al. 2013, Davis 2015, FAO 2018). Fishmeal provides B vitamins, minerals (Phosphorus, Calcium and trace elements), unidentified growth factors and n-3 long chain polyunsaturated fatty acids (LC PUFA) (Davis 2015). The bulk of fishmeal is manufactured from fish caught for meal and oil production, The following are the major species used for fishmeal production with sardines, capelin, menhaden, anchovy, mackerel and herring being the most important (Bowyer et al. 2013, Davis 2015).

The global production of fish meal remained relatively constant since the end of the 1980s with production of nearly 6 million metric tons per year. The competition and demand for this resource has increased over the years even though its supply has remained static. Bovine spongiform encephalopathy (mad cow diseases) is reported to be one of the main reason fishmeal is in high demand with reduced utilization of other bone meals and other meat (Trushenski et al. 2011). However, Asche and Tveterås (2004) reported that the increased utilization of fishmeal was mainly because of low cost per unit protein. Fishmeal, because of its growing demand is expected to separate from oil meat and stand as unique product (Trushenski et al. 2011).

The global increase of the aquaculture industry has exerted pressure on fishmeal demand. This has resulted in increased prices of fishmeal over the last several decades making it hard for fish farms to continue using it as the main ingredient of proteins for aquaculture feeds (Webster et al. 1992, Francis et al. 2001, Bowyer et al. 2013). For this reason, high prices and short supply,

the use of fishmeal as primary ingredient of protein has decreased over the years. Aqua culturists have little alternative but to reduce the amount used or replace it with alternative protein sources (Jirsa et al. 2011, Bowyer et al. 2013, Buentello et al. 2015, Herman and Schmidt 2016).

Alternative protein sources

The alternative protein sources should be at least be cheaper than fishmeal and also meet certain nutritional requirements to qualify as replacement of fishmeal as protein source (Bowyer et al. 2013, Webster et al. 1992) . These nutritional requirements include, high nutrient digestibility, high protein level with balanced amino acid profile and acceptable palatability (Gatlin et al. 2007, Bowyer et al. 2013). The search for new protein sources is difficult because each species have their own unique nutritional requirements (Trushenski et al. 2011).

The use of plant feed ingredients have been deemed suitable replacement of fishmeal in the future of aquaculture industry for finfish production(Gatlin et al. 2007, Trushenski et al. 2011). Advantage of replacing animal products with plant protein is that plant protein is much cheaper (Trushenski et al. 2011). These plant proteins must however produce healthy fish diets at minimal cost while producing growth of finfish at high optimal growth rate(Gatlin et al. 2007). Alternative plant protein sources include soybean meal, cottonseed meal, rapeseed meal, canola meal and sunflower meal (Cai et al. 2022)..

Soybean protein is an example of a plant protein source that has been used in the aquaculture industry as replacement of fishmeal in aquafeeds (Francis et al. 2001, Gatlin et al. 2007, Buentello et al. 2015, Herman and Schmidt 2016, Webster et al. 1992) as it has a high content ranging from 44 to 48% and is cheaper than fishmeal (Cheng et al. 2003). Its amino acid profile is also well balanced when compared to other plant protein sources (Zhou et al. 2005). However, the high inclusion levels of soybean meal in finfish diets have resulted in decreased

growth (Webster et al. 1992, Gatlin et al. 2007, Trushenski et al. 2011) and health problems (Trushenski et al. 2011).

Zhou et al. (2005) showed that fishmeal replacement levels of up to 40% with soybean meal was successful in terms of growth but health of juvenile cobia, *R. canadum* deteriorated. Catacutan and Pagador (2004) working on red snapper, *Lutjanus argentimaculatus* showed decline in liver health when fish were fed 36 to 48% fishmeal replacement with soybean meal. An issue with plant feed stuff is that they are deficient in certain essential amino acids such as methionine and lysine, containantinutritional factors (ANFs) (Craig and Helfrich 2017) and high in lipid or ash content which reduces feed digestibility (Kureshy et al. 2000).

Like soybean meal, canola meal is used as an alternative plant protein source in the diet of aquaculture species (Ranjan and Athithan 2015). According to Sajjadi,and Carter (2004), canola meal has a superior amino acid profile compared to soybean meal and is cheaper than fishmeal. Similar to other plant protein sources, canola meal has ANFs such as phytic acid, glucosinolates, indigestible carbohydrates and phenolic constituents which can negatively affect health status and growth of the fish. However, because of its high protein content, availability, amino acid profile and the high price of fishmeal, an interest in canola meal as an alternative protein source for aquaculture feeds has grown (Gatlin et al. 2007).

Previous studies have showed that a blend of plant and animal protein sources can adequately replace a significant proportion of fishmeal in the diet of carnivorous species (Koch et al. 2016, Cabano 2017). Alternative animal protein sources that have been used around the world include, poultry by-product meal (PBM), meat and bone meal (MBM), bacteria protein meal (BPM) blood meal (BM) and feather meal (Ferouz et al. 2012). Poultry by-product meal (PBM) has a high protein content of 60%, a balanced amino acid profile, a steady sustainable supply exists and it is relatively cheaper than fishmeal (Hu et al. 2008). Seo et al. (2022) showed that

a blend of PBM, tuna by-product meal, soybean meal, black soldier larvae meal and black soldier fly oil can replace 35% of fishmeal without compromising growth in the diet of carnivorous olive flounder, *Paralichthys olivaceus*.

Blood meal is regarded as one of the important animal protein sources for aquaculture feeds because of its high-quality protein content (90 – 95% protein), high digestibility of 80 to 99% and being a good source of essential amino acids (Bureau et al. 1999, El-Harounand and Bureau 2007, Millamena 2002). A study by Haned et al. (2017) showed that up to 35% fishmeal can be replaced by blood meal without compromising growth on the diet of juvenile silver pompano, *Trachinotus. blochii*. A previous study by Jamil (2007) showed that 23% of fishmeal in the diet of red snapper, *L. argentimaculatus* can be replaced with blood meal without affecting growth. Further research on the partial and full of replacement of fishmeal with alternative cheap and sustainable protein sources in the diet of carnivorous species is need.

Advantages and disadvantages of aquaculture methods

One of the challenges that fish farmers face includes frequent disease outbreaks (Zhang et al. 2011, Bregnballe 2015). The risk of disease spreading in traditional farming such as ponds and cages is maximized as water is taken from the sea or river (Bregnballe 2015). Legislations and regulations of the use of environment are increasing which makes it hard to access water and land. Another disadvantage of using traditional farming methods is that the systems are entirely dependent on external environmental conditions such as cleanliness of water, water temperature and oxygen levels (Bregnballe 2015). Therefore, an aquaculture farming technique that eliminates or reduce these challenges is preferred. Recirculating Aquaculture System (RAS) seems to be the most effective solution that allows for a better control these problems (Zhang et al. 2011).

Research on the optimization of diet for *S. lalandi* has been done around the world (Matsunari et al. 2005, Bowyer et al. 2013, Le and Fotedar 2013) as well as in South Africa for cage culture (Dunn 2014). Several diets have been formulated for aquaculture of *S. lalandi* in cages but no diets have been specifically formulated for RAS. FAO (2015) describes RAS as land-based farming technique for aquatic organisms that re-uses water in the production process. Recirculating Aquaculture Systems (RASs) also offer less economic and environmental risks compared to the traditional farming techniques such as cages (Zhang et al. 2011, Orellana et al. 2014, Bregnballe 2015).

Advantages of RAS include an improved disease management, improved nutrient recycling and waste management, biological pollution control and better hygiene (Orellana et al. 2014, Bregnballe 2015). Generally, the feeding behavior of fish is largely influenced by environmental factors such as dissolved oxygen, water temperature, total ammonia nitrogen and nitrite. The improved water quality in the RAS may thus have an impact on the increased feed rates (Zhang et al. 2011). The improved water quality in RAS can be maintained throughout the production cycle and for that reason, contributes to an improved feed conversion ratio (FCR), health and growth of the fish (Zhang et al. 2011).

Another advantage of RAS is that the farmer has complete control of all environmental parameters. The control of environmental conditions such as oxygen content, water temperature, pH and salinity provide stable conditions that are favorable for the cultured species, resulting in minimal stress and better growth (Balami 2019). RAS also offers the advantage of saving water, thus decreasing nitrate concentration by de-nitrification (Zhang et al. 2011) which is harmful to fish (Green 2009, Woolley 2009).Therefore, RASs are a viable aquaculture method for carnivorous marine finfish in South Africa and thus the study of dietary protein requirementsand fishmeal replacement in the diet of juvenile yellowtail in RAS is vital if the industry is to expand to its full potential.

Although RASs are viable aquaculture method for farming carnivorous marine fish, the high protein requirement of carnivorous species has a negative impact on the water quality. Excess dietary protein is one of the factors that contribute to the buildup of waste in the water through fish excretion(Brunty 1997). The waste produced by fish builds up forming ammonia which can be toxic to fish in small amounts. This results in fish mortality requiring more water to be flushed out to lower ammonium levels. Lowering dietary protein level can help minimize buildup of ammonia in the waterbut low levels have resulted in poor growth (Sitek 2020). It is therefore important to determine the optimal dietary protein level of cultured species in a RAS.

Research Aim

The aim of this study was to optimize the protein requirements and evaluate the effectiveness of fishmeal alternatives in the diet of juvenile yellowtail kingfish, *Seriola lalandi*, reared in a Recirculating Aquaculture Systems (RAS).

Objectives

1. Determine the effect that dietary protein levels have on the (i) survival, (ii) growth, (iii) feed conversion ratio and (iv) health of juvenile yellowtail, *Seriola lalandi.*
2. Determine the effect that replacement of fishmeal with alternative protein sources has on the (i) survival, (ii) growth, (iii) feed conversion ratio and (iv) health of juvenile yellowtail, *Seriola lalandi.*

Research hypotheses

1. Different dietary protein levels will have an effect have on the (i) survival, (ii) growth, (iii) feed conversion ratio and (iv) health of juvenile yellowtail, Seriola lalandi.

2. Different fishmeal replacement levels will have an effect have on the (i) survival, (ii) growth, (iii) feed conversion ratio and (iv) health of juvenile yellowtail, Seriola lalandi.

CHAPTER 2

GENERAL MATERIALS AND METHODS

Research experiment system

The study was conducted at the marine finfish farm, Kingfish Enterprises (Pty) Ltd in the East London Industrial Development Zone (ELIDZ). A purpose designed Recirculating Aquaculture System (RAS) was built for the experiment (Figure 2.1 and 2.2). The system consisted of twelve 8000 L circular tanks and a filtration system (with sump (1890 L), foam fractionator, sand filter and biological filter (4500 L) (Figure 2.1 and 2.2). The entire volume of water was exchanged in each tank every two hours. Filtered, unused seawater was pumped into the biofilter to replace 5-10% of the system's volume every day. Each of the experimental tanks were supplied with water inlet pipes and a single airstone supplied with air. The overflow of the water from each tanks drained into the sump. Water from the sump was partially directed through a protein skimmer into the moving-bed biological filter and was pumped through a sand filter before being pumped back to the tanks. The sand filter was backflushed two times per day (water flow in the tanks was not compromised) and sediments on the bottom of the tanks and sump were siphoned three times a week.

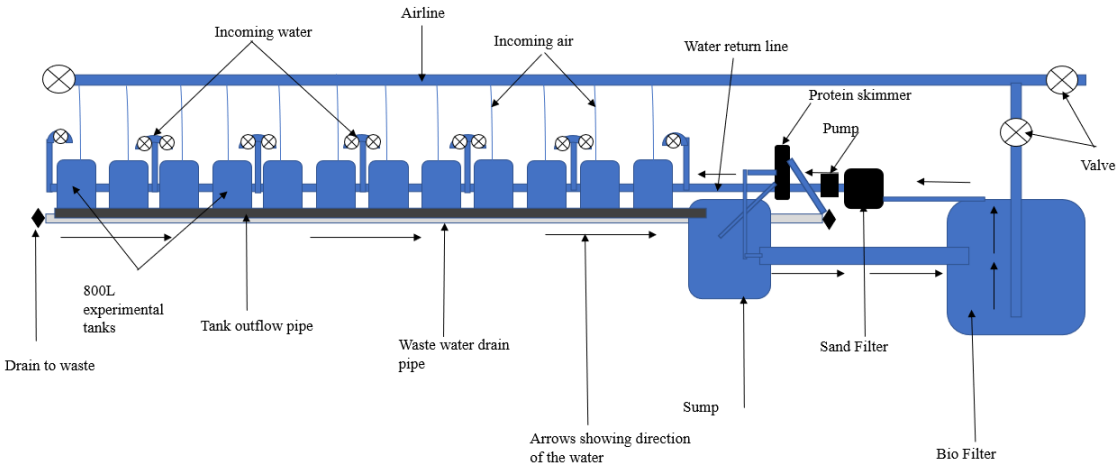

Figure 2.1: Section plan of the research experiment Recirculating Aquaculture System (RAS) constructed at Kingfish Enterprises in East London IDZ.

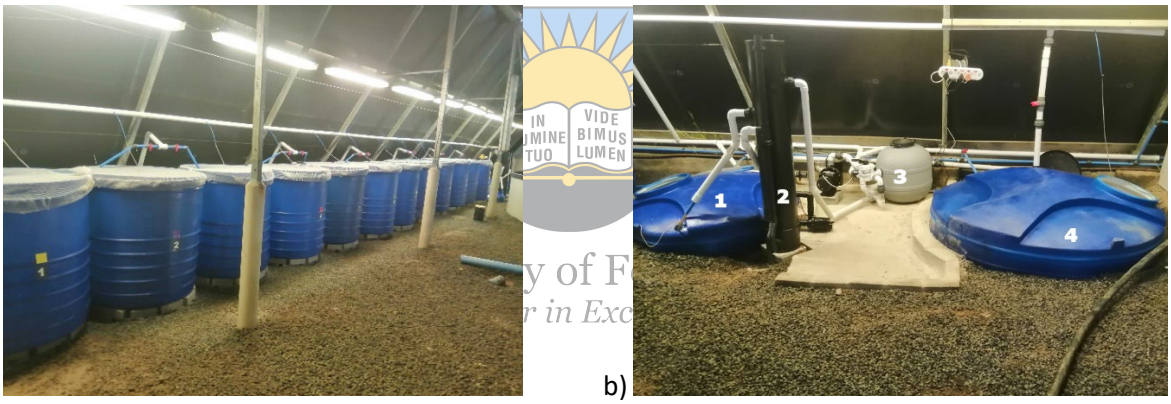

Figure 2.2: a) 800 L tanks in the research experimental system and (b) filtration system consisting of sump (1), foam fractionator (2), sand filter (3) and biological filter (4).

Temperature (°C), pH, dissolved oxygen (% saturation), dissolved oxygen (mg/L) and total ammonia nitrogen (TAN) were measured daily while Nitrate (mg/L) was measured once a week. The toxic ammonia (NH_3) portion was calculated using TAN, temperature and pH. The average values were recorded and presented in their respective chapters.

Experiment animals

Juvenile yellowtail (*Seriola lalandi*) spawned and reared by Kingfish Enterprises (Pty) Ltd were donated to the project. Fish were initially fed with commercial yellowtail Aqua Management Technologies (AMT) diet currently being used at the Kingfish Enterprises before being donated to the project. The AMT diet consisted of 47.6% protein, 10.6% lipid, 17.3% carbohydrates and 13.07 Mg/kg energy.

Prior to the start of the experiment, fish were starved for 24 hours. At the beginning of the experiment, fish was anaesthetised using 0.2 mL/L 2-phenoxyethanol and once sedated were individually weighed (g) and measured (mm).

The fish were fed to satiation two times a day. Satiation was assumed when fish stopped feeding at the surface.

Diet formulation and manufacturing

The experimental diets were formulated with the assistance of Professor Cliff Jones (Department of Ichthyology and Fisheries Science, Rhodes University) and manufactured by Marifeed (Pty) Ltd. factory in Hermanus, South Africa. Fishmeal was used as the only protein source for the dietary protein level experiment (Chapter 3) whereas a blend of fishmeal, blood meal, poultry meal and canola meal (rapeseed) were used as the protein sources for fishmeal replacement experiment (Chapter 4). Marine fish oil (Oceana (Pty) Ltd, Cape Town, South Africa) was used as the lipid source whereas vegetable starch was used as the source of carbohydrates.

The dry ingredients were weighed and thoroughly mixed and water and fish oil were then added to the mixture to make a homogenous dough. Preparatory technologies developed for the

abalone-farming industry were used to extrude feed through a circular 4.0 mm die and pellets were then cut at 3 mm long. The pellets were then dried at 38 °C for 16 hours, cooled to room temperature and packaged. The formulated feeds were then stored at -20°C in sealed packets (Jones et al. 2021).

Individual containers of food were assigned to each tank and were weighed at the start of the experiment and after each week of feeding. Feed intake was measured by substituting feed left in the container from the initial feed weight of the feed inside the container. Each dietary treatment (in all experiments) was replicated three times (3 tanks per treatment).

Proximate composition analysis

At the end of each of the two experiments, two randomly selected fish from each tank were anaesthetised using 2-phenoxyethanol and killed by pithing before being frozen for analysis. Fish were cut up and dried in an ExoTherm digital oven for 24 hours at 70°C. After drying, fish were then ground by pestle and mortar. Proximate composition of fish and feed were then analysed by the Soil Fertility and Analytical Services at KwaZulu Natal Department of Agriculture and Rural Development.

Proximate analysis was also undertaken on all experimental diets and fish and are presented in their respective chapters. The following methods were used:

Protein

The Micro-Kjeldahl method was used to determine protein content (Jobling, 2001):

Approximately 100 g of sample was weighed into digestion flasks. Concentrated sulphuric acid (2.5 ml) and Selenium catalyst (2.5 g) including the blanks were added to each flask, Pre-heated block was used to put on the flasks and 1 ml hydrogen peroxide was used to wash them after

10 minutes for 60 minutes. The digestion flasks were then left for another 20 minutes just before the final wash was completed and then removed from the block. The same number of 200ml Erhlenmeyer flasks were arranged while the flasks were left to cool. An indicator with 10 ml of 1% boric acid mixture was pipetted into each flask. In each digestion flask, 10 ml of distilled water was added and a clean steam-distillation flask was used for the transfer of contents. A 10ml caustic/hypo mixture was used to rinse the flasks and were then transferred to distillation flask. This was then positioned in the Steam- distillation apparatus and distilled for seven minutes. Erhlenmeyer flasks (200ml) were prepared and set up in the collecting position so that the tip of the delivery tube was submersed in the boric acid solution. The solution which resulted from the Erhlenmeyer flask was titrated with the standard 0.015M HCl solution to the grey end point. The volume of HCl titrated was used to calculate the percentage using the following formulae:

N = (M HCl * 14.007 * 100)/ weight of sample (mg)

The percentage protein was calculated as: N x 6.25

Fat

The method described by Knauer et al. (1994) which was modified from the chloroform-methanol extraction method (Folch et al. 1957) was used to determine fat content.

Centrifuge tubes were used to measure five 0.2g samples of powdered feed and fish and rehydrated with 3ml distilled water. A 6.25ml of chloroform and 6.25 of methanol were added to each of these solutions and were then homogenized for 2 minutes, after 6.25ml of distilled water was added. The solutions were homogenized for another minute and then centrifuged at 3000g for 10 minutes. Pipette was used to pipette 0.75ml of the bottom layer from each of the solutions into dried, clean crucibles of know weighed and evaporated to dryness on a hot plate. The oven was pre-heated at 100°C and crucibles were then placed for 30 minutes Desiccator

was then used to cool the samples before being weighed. Percentage fat was then calculated using the following formula:

$$\% \text{ Fat} = ((\text{mass of fat (g)} * 25/15)/ \text{ mass of sample (g)}) * 100$$

Moisture

Three 1 -gram samples were weighed before and after drying at 70°C until a constant weight was achieved.

Ash

Three 0.5g powdered samples were first died at 70°C and then burned in open crucibles in a muffle furnace at 550°C for 7 hours. Desiccator was then used to cool the samples before being weighed.

Energy

The calorific value (CV) of the diets determined the energy content and CP400 Calorimeter systems apparatus was used for measuring. A standard calorific value of 26.454 MJ/kg was used to calibrate the machine using CV of 0.5g Benzoic acid. Samples were then weighed, dried and placed individually into the bomb. Pure oxygen was pumped into the bomb at a pressure of 30 bars prior to ignition. The calorific value (CV) was measured in MJ/kg and read after ignition.

Hepatosomatic index (HSI)

Two fish from each tank were euthanized as explained in the proximate composition analysis section, weighed, dissected and liver samples were removed and weighed to calculate the HIS using the following formula:

HSI = [liver weight (g)/ total body weight (g)] x 100

Histological preparation and analysis

Two fish used from each tank to determine HIS had samples of their liver removed which were fixed in 10% buffered formalin for histological examination. Liver samples were then sent to African Aquatic Vet Services (Pretoria) for preparation of slides. Fractions of each sampled liver was dehydrated in a graded ethanol series and then embedded in paraffin wax. Haematoxylin and Eosin (H&E), periodic acid-Schiff diastase (PASD) and periodic acid-Schiff (PAS) were used to stain four µm sections of the liver for light microscopy examination (Genten et al. 2009).

Examinations of slides were performed using a BM2000 Microscope at magnification of 40X. Hepatocyte nuclei were considered normal if the shape was spherical, with a central nucleoli and abnormal if the nucleus was shrunken and nucleoli was not central. One hundred Lipid vacuole zones were measured on each liver section (Rossetti 2012).

Statistical analysis

Percentage weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) were all calculated using the following formulars.

% Weight gain = [(final weight (g) – initial weight(g)) ÷ initial weight(g)] × 100

SGR = [Ln (final weight(g)) – Ln (initial weight(g))] x 100 ÷ time (days)

CF = 100 × whole body weight (g) ÷ (body length [cm])$^{2.84}$

FCR = dry feed supplied(g) ÷ wet weight gained (g)

PER = body weight gain (wet weight g) ÷ protein ingested (g)

Normality of the data was checked using a Kolmogorov-Smirnov test while the homogeneity of variances was checked using Levene's Test. Where both assumptions were met, a One-way ANOVA was used to compare dietary treatments. Tukey's multiple range test was used to identify differences between individual diets. Significance was set at $p< 0.05$.

CHAPTER 3

THE EFFECT OF DIETARY PROTEIN LEVEL ON SURVIVAL, GROWTH, AND FOOD CONVERSION RATIO OF JUVENILE YELLOWTAIL IN A RECIRCULATING AQUACULTURE SYSTEM (RAS)

Introduction

In intensive aquaculture systems, feed is considered as one of the largest costs of farming fish contributing from 42% to 50% of the operating costs of farming fish from juvenile to market size (Irish 1997, Bowyer et al. 2013). For growth, protein is one of the most important ingredient in the feed of fish, and generlly the most expensive (Daniel 2004, Woolley 2009). Protein contributes about 65-75% of the dry matter in the tissues of fish compared to other nutrients making it the most important of them all (Bowyer et al. 2013). It is therefore crucial that the fish obtain adequate levels of protein to ensure good survival, optimal growth and good food conversion ratio (FCR) while not over supplying expensive protein which is being wasted.

When provided with insufficient dietary protein, fish will metabolise protein from less vital tissues of the body to maintain the functions of more vital body functions (Wilsom 2003). This results in poor survival and a reduction in the growth and weight of the fish (Wilsom 2003, Davis 2015). It is therefore important to optimize protein levels which will result in the best growth at the lowest cost (Irish 1997, Gordon 1999, Woolley 2009).

It is important to know the protein requirements of the fish so that the protein is spared from being used as energy source (Bowyer et al. 2013). Protein requirement is the amount of dietary protein that an animal needs to achieve maximum growth (Bowen 1987). When fish are provided more protein than they need, the protein is used for energetic requirements (De Silva and Anderson 1994) which is unnecessary as cheaper sources of energy such as lipids and

carbohydrates can be used instead (Davis 2015). Excess protein and imbalanced amino acids found in protein also results in excess nitrogenous waste into the surrounding water which is harmful to fish. Excess nitrogenous waste decreases feed efficiency in fish, resulting in poor survival and growth (Bowyer et al. 2013).

The major requirement for any formulated diet of finfish species is to maximize growth (Steffens 1989). Most aquaculture diets for carnivorous species are high in protein and therefore supply essential amino acids which are responsible for growth (Woolley 2009). Fishmeal has typically been the main ingredient when formulating diets for carnivorous fish because of its high protein content, good digestibility and essential fatty acids (Davis 2015, FAO 2018). Fish from the genus *Seriola* are carnivorous and therefore require high protein levels in their diet for optimal growth (Moran et al. 2010, Stuart et al. 2018).

Determination of protein requirement for farmed species in a RAS is important because of the impact it has on water quality. One of the issues farmers have with the use of RAS is the waste produced by fish because of excess protein (Wheaton 2002, Tom et al. 2021). Fish excrete nitrogen which comes from excess protein as ammonium ions which can be toxic to fish at low amounts (Randall and Wright 1987, Ip and Chew 2010). High levels of ammonia present in water will limit one the most important benefits of RAS which is the reuse of water. Water will then need to be exchanged at a higher rate to maintain acceptable levels of toxic ammonia. Although low levels of protein have resulted in improved water quality, poor or reduced growth have been reported (Sitek 2020). It is therefore vital to optimize protein requirement of the species cultured in RAS to minimize protein waste while ensuring optimal growth. **

The aim of this study was to determine the dietary protein level of juvenile yellowtail, *Seriola lalandi* in a RAS which optimized survival, growth, FCR and health.

The objectives of the study were to assess the (i) survival, (ii) growth and (iii) feed conversion ratio and (iv) health of juvenile yellowtail fed formulated feeds containing different protein levels in a RAS.

Materials and methods

Experimental animals

Juvenile yellowtail (*S. lalandi*) spawned and reared by Kingfish Enterprises (Pty) Ltd were donated to the project. Fish were initially fed with a commercial yellowtail diet (Aqua Management Technologies (AMT)) is currently being used at the Kingfish Enterprises. The AMT diet consisted of 47.6% protein, 10.6% lipid, 17.3% carbohydrates and 13.07 Mg/kg energy.

Prior to the start of the experiment, fish were starved for 24 hours. At the beginning of the experiment, fish were anaesthetised using 0.2 mL/L 2-phenoxyethanol and once sedated were individually weighed (g) and measured (mm). A total of 360 juvenile yellowtail were randomly assigned into twelve tanks with 30 fish per tank (weight 22.26 ± 4.94g and length 117.03 ± 8.79mm).

For the experiment, fish were fed to satiation two times a day, satiation was assumed when fish stopped feeding at the surface. Individual containers of food were assigned to each tank and were weighed at the start of the experiment and after each week of feeding.

Experimental system

The experimental RAS described in Chapter 2 was used for this experiment.

Water temperature (°C), dissolved oxygen (mg/L), pH and total ammonia nitrogen (TAN) were measured daily while nitrate (mg/L) was measured once a week. The toxic ammonia (NH_3) portion was calculated using TAN, Temperature and pH.

Experimental system and stocking condition

The experimental system described in Chapter 2 was used for this experiment.

Prior to the start of the experiment, fish were starved for 24 hours. At the beginning of the experiment, fish was anaesthetised using (0,2 ml/L) 2-phenoxyethanol and once sedated were individually weighed (g) and measured (mm).

Water temperature (°C), dissolved oxygen (mg/L), pH and total ammonia nitrogen were measured daily while nitrate (mg/L) was measured once a week.

Dietary treatments

Four experimental diets with four different protein levels were formulated guided by the literature (Thomas et al. 1991, El-Dahhar et al. 2006, Ramezani 2009, Seo et al. 2022) and Kingfish Enterprises diet. Protein levels were set at 38%, 44%, 50% and 56%. Lipid levels were set at 18.45%, 17.19%, 15.92% and 14.65% to balance energy (19.50 MJ/kg). The experimental diets were formulated with the assistance of Professor Cliff Jones (Rhodes University) and manufactured by Marifeed (Pty) Ltd. factory in Hermanus, South Africa. Low-temperature, formaldehyde free, fishmeal (66 % crude protein, 8.0 % lipid, Oceana (Pty) Ltd, Cape Town, South Africa) was used as the main protein source. Marine fish oil (Oceana (Pty) Ltd, Cape Town, South Africa) was used as the lipid source whereas vegetable starch was used as the source of carbohydrates (Table 3.1).

The four experimental diets were randomly assigned to three replicate tanks for the duration of the experiment.

Table 3.1. Formulation and proximate composition of the experimental diets (g/10 g dry weight).

Ingredients	**38%**	**44%**	**50%**	**56%**
Fishmeal	5.39	6.38	7.37	8.36
Starch	3.33	2.54	1.75	0.95
Vitamix	0.01	0.01	0.01	0.01
Marine Fish Oil	1.27	1.07	0.87	0.68
Total (dry weight) (g/10g)	**10.00**	**10.00**	**10.00**	**10.00**
Lipid** (%)	18.45%	17.19%	15.92%	14.65%
Energy** (MJ/kg)	19.50	19.50	19.50	19.50
Protein** (g): Energy (MJ/kg)	1.95	2.26	2.56	2.87
Methionine (%, g meth. /100 g diet)	1.05	1.22	1.39	1.56
Methionine (%, g meth. /100 g protein)	2.76	2.77	2.78	2.79
Lysine (%, g lysine/100 g diet)	2.75	3.22	3.70	4.17
Lysine (%, g lysine/100 g protein)	7.23	7.32	7.39	7.45
Analyzed Composition				
Crude protein (% dry matter)	38.83	45.31	50.80	56.85
Crude lipid (%)	17.15	16.31	16.65	15.91
Ash (%)	12.18	10.95	12.57	14.38
Moisture	7.01	7.72	6.54	7.42

**Calculated

The experiment ran for total of 49 days with fish being sedated, weighed (g) and measured (mm) on days 0, 21, and 49.

Proximate analysis

Fish were cut up and dried in an ExoTherm digital oven for 24 hours at 70°C. After drying, fish were then ground by pestle and mortar. Proximate composition of fish and feed were then analysed by the Soil Fertility and Analytical Services at KwaZulu Natal Department of

Agriculture and Rural Development. The methods for proximate analysis are outlined in Chapter 2.

Histological preparation and analysis

The two fish used to determine HIS had samples of their liver which were fixed in 10% buffered formalin for histological examination. Liver samples were then sent to African Aquatic Vet Services (Pretoria) for preparation of slides Fractions of each sampled liver was dehydrated in a graded ethanol series and then embedded in paraffin wax. Haematoxylin and Eosin (H&E), periodic acid-Schiff diastase (PASD) and periodic acid-Schiff (PAS) were used to stain four µm sections of the liver for light microscopy examination (Genten et al. 2009).

Examinations of slides were performed using a BM2000 Microscope at magnification of 40X. Hepatocyte nuclei were considered normal if the shape was spherical, with a central nucleoli and abnormal if the nucleus was shrunken and nucleoli was not central. One hundred Lipid vacuole zones were measured on each liver section.

Statistical analysis

Percentage weight gain, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (CF) were all calculated using the following formulae.

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

$$\text{SGR} = [\text{Ln (final weight(g))} \div \text{Ln (initial weight(g))}] \times 100 \div \text{time (days)}$$

$$\text{FCR} = \text{dry feed supplied(g)} \div \text{wet weight gained (g)}$$

$$\text{PER} = \text{body weight gain (wet weight (g)} \div \text{protein ingested (g)}$$

The normal distribution of the data was checked using Kolmogorov-Smirnov test. The homogeneity in variances was checked using Levene's Test. A One-way ANOVA was used to

compare data from replicates within each dietary treatment for significant differences. Tukey's multiple range test was used to e differences among individual diets. Significance was set at $p< 0.05$.

Results

Water quality

Total ammonia nitrogen (TAN), Daily unionized ammonia (NH_3), temperature, nitrate, pH, dissolved oxygen and percentage saturation of dissolved oxygen in experimental RAS are represented in Table 3.2.

Table 3.2: Water quality parameters averages maintained for the duration of the study.

Parameter	**Average**
Temperature (° C)	17.7±0.87
Dissolved oxygen (mg/L)	7.7±0.37
Dissolved oxygen (% saturation)	89.88±9.81
Total ammonia nitrogen (TAN)	0.52±0.24
pH	7.86±0.09
NH_3 (mg/L)	0.01±0.01
Nitrate (mg/L)	43±3.94

Survival

Survival was not affected by different protein levels during the study. Mortalities did, however, occur because of an overdose of anaesthetic to tanks 1 and 12 on day 21 during the weigh and

measure. Subsequent to this, 100% survival was recorded for the remainder of the experiment.

(

Growth performance

Overall, SGR was greater for fish fed 50 and 56% protein (One-Way ANOVA, F = 9.26 *df* = 3, p = 0.006) compared to fish fed 38% protein. There were however no differences in SGR between fish fed 44, 50, and 56% protein diets (Figure 3.1).

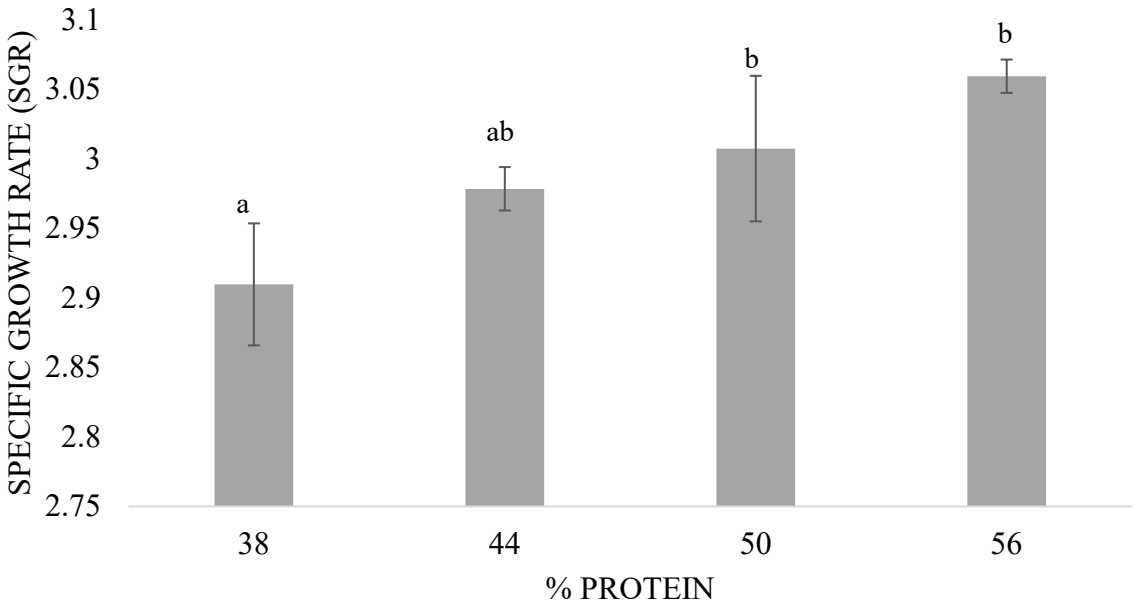

Figure 3.1: Average Specific Growth Rate (SGR) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being fed four protein levels. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$)

Fish fed 38% protein had the lowest average weight (One-Way ANOVA, F= 12.5 *df*=3, p= 0.002) and length (One-Way ANOVA, F= 18.8 *df*=3, p= 0.01) compared to fish fed 44%, 50% and 56% protein (Figure 3.2).

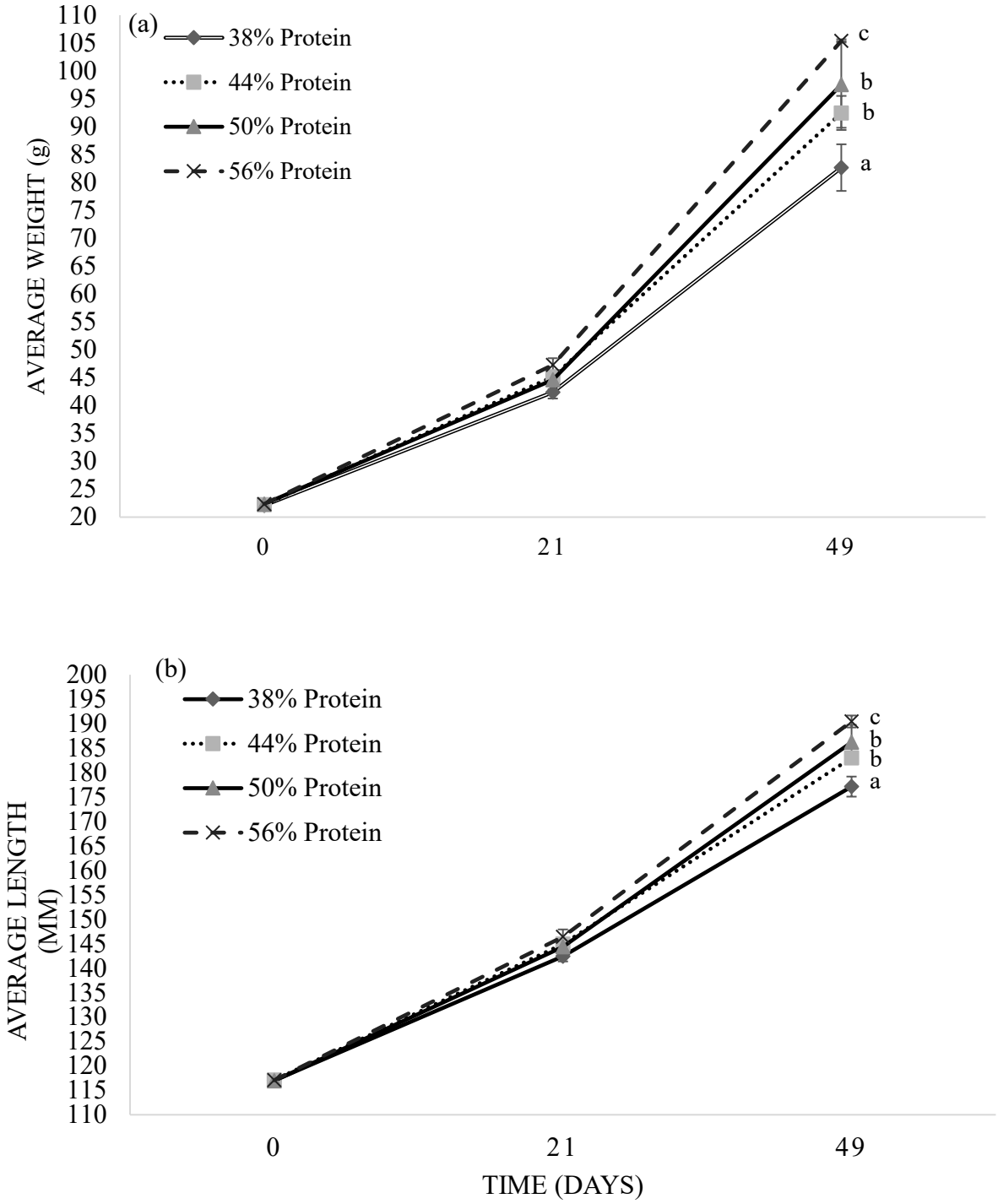

Figure 3.2: Average weight (a) and length (b) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being fed four protein levels. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

Feed efficiency

Feed conversion ratio was the same across all diets in the first growth period (day 0-21). In the second growth period (day 21-49), FCR was best for fish fed 44%, 50% and 56% protein compared to fish fed 38% protein (One-Way ANOVA, F=17.48 *df*=3, p= 0.001) (Figure 3.3).

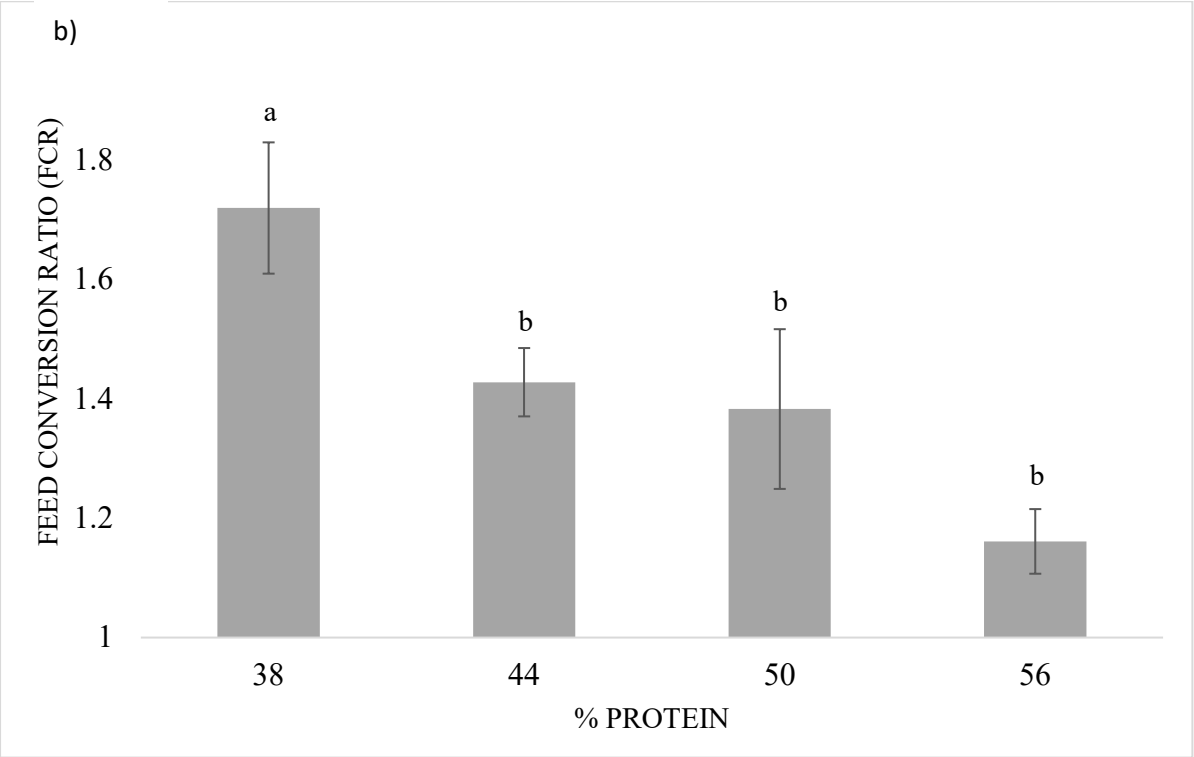

Figure 3.3: Feed Conversion Ratio (FCR) of juvenile yellowtail kingfish, *Seriola lalandi* at four protein levels (days 21-49). Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

Protein Efficiency Ratio (PER) was not affected by different dietary protein levels (Figure 3.5).

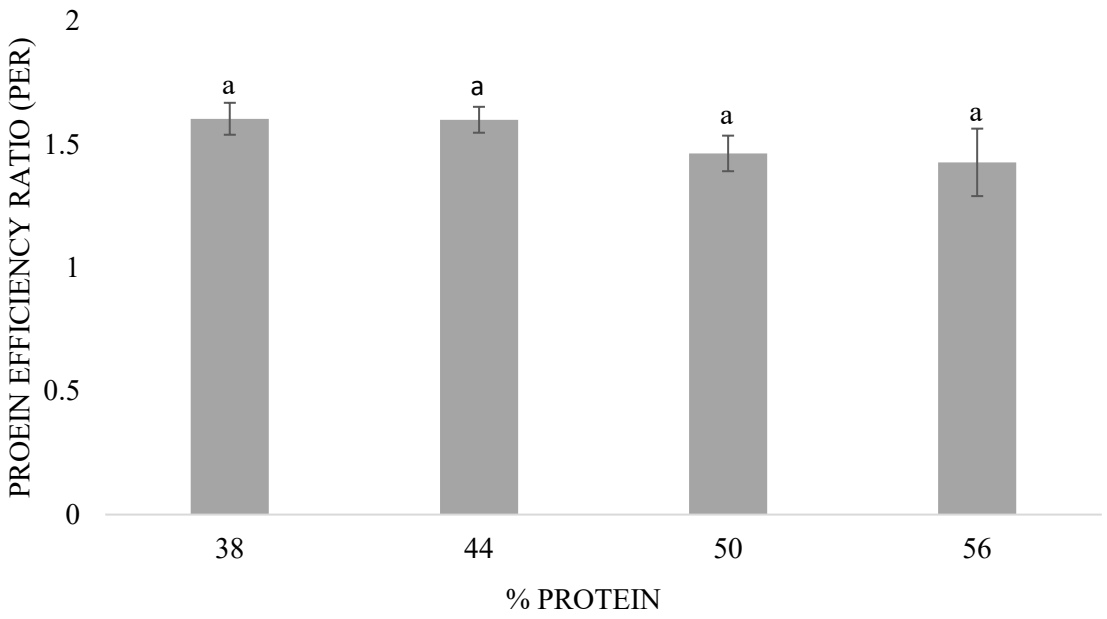

Figure 3.5: Protein Efficiency Ratio (PER) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being fed four protein levels. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

Table 3.3: The effect of dietary protein levels on weight gain, Specific Growth Rate (SGR), Food Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) of Juvenile yellowtail, *Seriola lalandi* fed after 49 days. Data are mean values ± standard deviation of three replicates.

Diets (% protein)	**38%**	**44%**	**50%**	**56%**
Parameter				
% Weight gain	274.08±23.22^{a}	315.93±11.08^{ab}	336.51±34.94^{b}	371.53±5.53^{b}
SGR	2.89±0.13^{a}	2.98±0.05^{ab}	3.01±0.16^{b}	3.06±0.02^{b}
FCR	1.76±0.2^{a}	1.42±0.05^{a}	1.64±0.48^{a}	1.26±0.09^{a}
PER	1.52±0.14^{a}	1.6±0.05^{a}	1.28±0.32^{a}	1.43±0.1^{a}

Values in each row with different superscripts indicates significant differences ($p< 0.05$).

Microscopic evaluation of the liver

The hepatocytes of fish fed high dietary lipid content (diet with 38% and 44% protein) showed large lipid vacuole zones in the cytoplasm. These treatments also showed spherical nuclei located in the centre of the cells which is an indication of healthy liver (Figure 3.6).

Fish fed high dietary protein (50% and 56% protein) with low lipid content (One-Way ANOVA, $F= 39.77$ $df=3$, $p< 0.001$), 15.92% and 14.65% respectively showed less prominent lipid vacuolated zones compared to fish fed high levels of lipid (38% and 44% protein diet) (Figure 3.6).

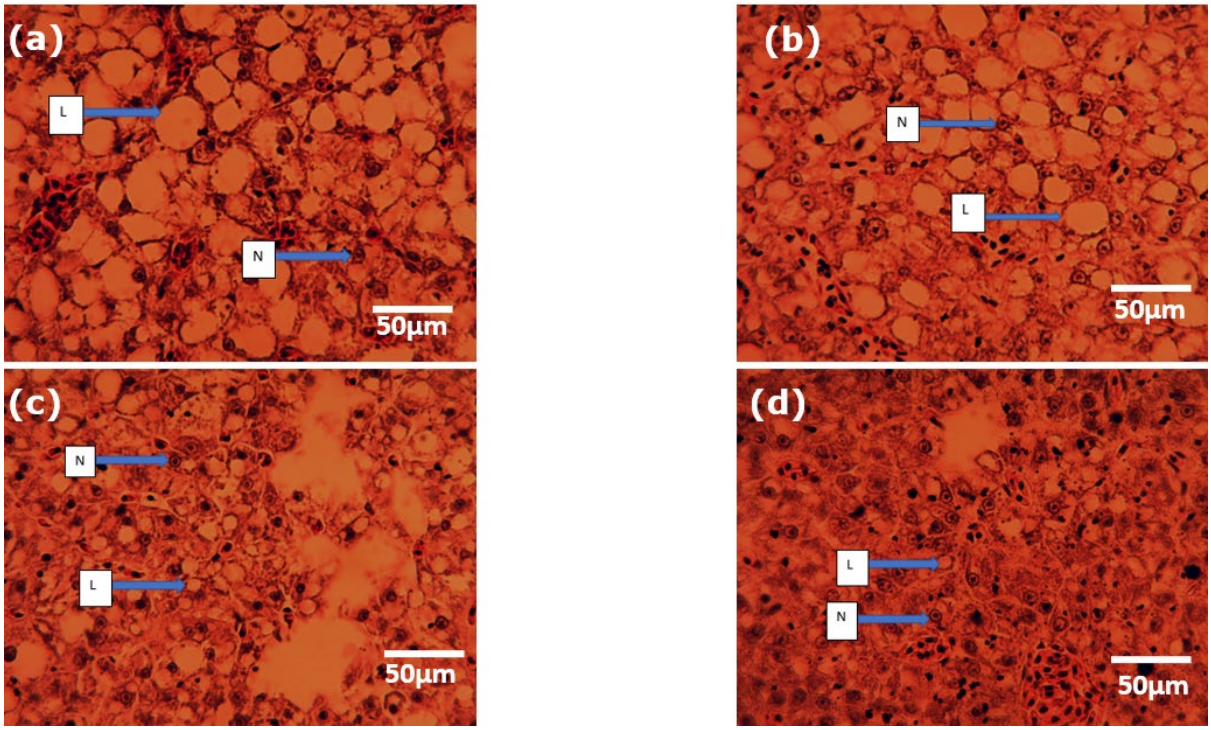

Figure 3.6: Liver section (400x) of *Seriola lalandi* fed (a) 38%, (b) 44%, (c) 50% and (d) 56% protein after 49 days. L represents lipid vacuoles while N represent nuclei.

Proximate composition analysis

Body protein, ash, moisture and lipid was not affected by different dietary protein levels (Table 3.4).

Table 3.4: Proximate composition of whole body of *Seriola lalandi* after 49 days of being fed four protein levels. Data are mean ± standard deviation of three replicates.

Diets (% protein)	**38%**	**44%**	**50%**	**56%**
Parameter				
Moisture (%)	6.26±0.86[a]	7.11±0.84[a]	7.69±1.82[a]	5.55±0.06[a]
Crude protein (%)	59.49±2.35[a]	61.14±0.01[a]	63.39±6.08[a]	61.01±1.13[a]
Crude lipid (%)	14.15± 3.17[a]	19.42±0.84[a]	20.88±3.10[a]	20.41±1.45[a]
Ash (%)	10.48±0.87[a]	9.81±1.15[a]	9.44±0.48[a]	9.77±0.13[a]

Values in each row with different superscripts indicates significant differences ($p < 0.05$).

Discussion

Survival

Survival is the most important biological factor that influences the successful farming of a species (Collet 2007, Tun and Swe 2019). In this experiment, different dietary protein levels did not have an effect on the survival of juvenile yellowtail.

Growth parameters

After survival, the main aim in aquaculture is to produce the highest growth at minimal cost, thereby ensuring profit ability (Irish 1997, Besson et al. 2020). An increase in growth rate is expected to increase profit due to a reduction of production time (Besson et al. 2020). The growth of juvenile fish is mostly depended on protein and out of all the nutrients protein is the

most expensive, therefore a careful attention must be paid to protein requirement (Irish 1997, Woolley 2009) .

A study by Dwyer et al. (2002), showed a high protein requirement of 55% for juvenile yellowtail flounder, *L. ferruginea*. The same is true for golden mandarin fish, *S. scherzeri* which require 55% protein (Sankian et al. 2017). Similarly, Daniel (2004) showed that juvenile dusky kob, *A. japonicas* required 45% protein. In this study, an increase in protein level resulted in increase in the weight gain and specific growth rate of juvenile yellowtail. A similar trend was observed for juvenile marbled flounder (*Pseudopleuronectes yokohamae*) where an increase in dietary protein level from 42.7 to 64.5% resulted in increase in % weight gain (Cho et al. 2021). Booth et al. (2010) suggested that the high protein requirement of fish may result from fish utilizing dietary protein to supplement their energetic requirements rather than growth.

Two trends of growth for carnivorous species have been observed for fish fed diets with different protein levels. The first trend shows an increase in growth with an increase in protein level up until it reaches a plateau, then a further increase will result in decline in growth Mazid et al. 1979). This trend has been found in dusky kob, *A. japonicus* where *A. japonicus growth* increased from 35% to 49.5% protein level and decreased at 49.5% protein level (Daniel 2004). Similarly, juvenile marbled flounder, *P. yokohamae* increased from 42.7 % to 58.8% protein and decreased after 58.8% protein (Cho et al. 2021). The second trend does not reach a threshold rather increasing in growth as protein increases. This trend was found in golden mandarin fish, *S. scherzeri* where growth increased from 35% to 55% protein level (Sankian et al. 2017). This study on *S. lalandi* followed the second trend where growth of increased with an increase in protein level and no threshold was reached. Since growth did not reach a plateau

over the different dietary protein levels which were tested in this study, it is likely that juvenile yellowtail can utilize protein at levels above 56% without negatively impacting growth. Pursuing studies on protein requirements above 56% would however not make any economic sense as growth at 44% protein was already at the same level as fish fed 56% protein.

Feed conversion ratio (FCR) is important in aquaculture because it helps determine the acceptability of diet and one of the main indicators of feed efficiency (David 2015, Tun and Swe 2019). The acceptability of diet depends on whether the diet improves the FCR and decreases environmental impacts resulting in increased profits (Besson et al. 2020). Overall, FCR was similar across all dietary treatments (Table 3.3). The reason could be lack of precision in feeding because of poor water clarity which made observation of the fish feeding difficult during the first growth period (day 0 to 21). In the second growth period (day 21 to 49) water clarity improved as well as feeding. Differences in FCR were then found in this period with FCR (1.26-1.76) decreasing with increasing dietary protein level. The decrease in FCR with increasing protein (25% - 45% protein) was found for juvenile (1.29-0.99) and pre-adult (20% - 45% protein) (1.73-1.16) gibel carp (*Carassius auratus gibelio*) (Ye et al. 2017) and juvenile marbled flounder (*Limanda ferruginea*) (1.92-1.22) (Cho et al. 2021). The FCR values found in this experiment (1.26-1.76) falls within FCRs values found for other carnivorous species but are lower than FCR values found by other authors for other carnivorous fish species at similar size. A study by Maldonado-García et al. (2012), found FCR value of 2.36-4 for yellowtail snapper while Daniel (2004) showed FCRs of 1.26-1.78 for juvenile dusky kob. The lower FCR ranges obtained in this experiment indicate efficient food utilization by juvenile yellowtail. Lower FCR values were also found by Booth et al. (2010) and it was suggested that it could be the result of high growth in fish fed high level of dietary protein.

The PER was not affected by different protein levels in the diets, contradicting previous studies of other carnivorous species. Previous studies of carnivorous species have shown that an increase in protein level results in a decrease in PER. Findings by Coutinho et al. (2016) for zebra sea bream, *D. cervinus* and Maldonado-García et al. (2012) for yellowtail snapper (*L. argentiventris*) exhibited similar trend where PER decreased with increasing protein levels. The PER values (1.6-1.76) found in this experiment are lower than the reported range of PER for *D. cervinus* (1.09-1.9) (Coutinho et al. 2016) and *A. japanicasI* (1.02-1.69) (Daniel 2004). Rossetti (2012) reported that low PER values show that fish could not efficiently utilize protein to support growth.

Microscopic evaluation of the liver

Liver histology in fish nutrition studies is important because it assess the health status of the fish (Raskovic et al 2011). In assessing health status of the liver, lipid content in the cytoplasm and hepatocytes nuclear area are considered (Rossetti 2012). Diets with 38% and 44% protein exhibited high levels of lipid vacuolated zones in the cytoplasm compared to high protein diets, 50% and 56% protein. The reason could be physiological response due to excess dietary lipid and subsequent storage by liver as an energy storage (Mosconi-Bac 1987). Several authors have reported that high levels of lipid in the liver represents a well-fed status rather than a pathological syndrome (Segner and Witt 1990, Kaushik 1997). In terms of hepatocyte nuclear area, no differences were observed among different diets. Tacon, (1992) reported that histological changes in the liver (such as shape and location of the nuclei) are easily recognized if the food is not adequate. None of those were identified in this study, suggesting that the dietary formulations were nutritionally balanced and therefore met the dietary needs of *S. lalandi*.

Proximate composition

Different dietary protein levels did not affect body protein, lipid, ash and moisture of *S. lalandi* in this experiment. Similar results were reported for *S. scherzeri* (Sankian et al. 2017), *S. senegalensis* (Valente et al. 2011) and *S. dumerli* (Vidal et al. 2008). Woolley (2009) and Maldonaldo-Garcia et al (2012) found contradicting results *A. japanicus* and *L. arbentiventris* respectively where body composition was affected by various protein levels in the diet. Reason for this could be due to variation in experimental conditions or fish species (Sankian et al. 2017).

Conclusion

The high cost of feed in aquaculture operations has led to detailed research on diet formulations that produce the best growth at the lowest cost. It is, however, vital that the formulated diets have all the essential nutrients that will aid in producing the best possible growth and survival of the fish at minimum cost (Woolley 2009). In juvenile *S. lalandi*, weight gain and SGR were significantly high for fish fed high protein diets (50% and 56%) compared to fish fed 38% protein but there were no significant differences in SGR and weight gain between high protein diets and 44% protein diet. Optimal (i.e., lower) FCR values were found at 44% and 56% protein levels. The 44% protein level was found to be optimal for juvenile yellowtail in RAS as it produces the best growth and lowest FCR.

Although 44% protein level was found to be optimal for *S. lalandi* in this study, fishmeal was used as the only protein source. Fishmeal is a finite resource and is, with the growing terrestrial and aquaculture sectors, becoming more and more expensive. It is therefore important to look at replacing fishmeal in the *S. lalandi* with more sustainable and cheaper protein sources to maximize profitability of farming the species.

CHAPTER 4

THE EFFECT OF REPLACING DIETARY FISHMEAL ON THE SURVIVAL, GROWTH, FCR, AND HEALTH OF JUVENILE YELLOWTAIL, *SERIOLA LALANDI* IN A RECIRCULATING AQUACULTURE SYSTEM (RAS).

Introduction

Fishmeal has typically been the main ingredient when formulating a diet for carnivorous fish because of its high protein content, good digestibility and essential fatty acids (Hertrampf 2003, Olsen and Hasan 2012). However, with the growth of aquaculture sector it is becoming unsustainable and highly expensive (Egerton et al. 2020). Aquaculturists have little alternative but to replace or reduce the amount of fishmeal in diets with alternative, more sustainable and less costly protein sources which improves the profitability of farming carnivorous species such juvenile yellowtail (*S. lalandi*).

Several studies have investigated the replacement of fishmeal with cheaper plant and animal sources for carnivorous species (Yigit et al. 2006, Collins et al. 2013, Egerton et al. 2020). Soybean meal is one of the common plant protein sources that have been used around the world to replace fishmeal in fish diets because of its cheap cost, high protein levels, and good balance of essential amino acids (Hertrampf and Piedad-Pascual 2000, Meng et al. 2019, Pervin et al. 2020). However, the issue with soybean meal is the antinutritional factors present such as phylate, lectines, saponines and indigestible carbohydrates (Lall and Anderson 2005, Xu and Hanna 2011). A study on Atlantic salmon (*S. salar*) where fishmeal was replaced with plant protein resulted in alteration of microbial composition resulting in poor health and growth of the fish (Egerton et al. 2022). Another study by Chen et al. (2019) showed that pearl gentian grouper (*E. lanceolatus*) was not able to fully utilize soy protein concentrate as the main source of protein with diets of more than 30% inclusion.

Like plant protein sources, animal protein sources are regarded as suitable alternatives to replace fishmeal because of their high protein and lipid content, balanced amino acid profile and low cost compared to fishmeal (Suloma et al. 2013). Several studies have indicated that fishmeal can be replaced by animal protein sources without compromising growth and survival (Hernández et al., 2014, Rossi and Davis 2014, Yang et al. 2022). A study on fishmeal replacement in the diet of juvenile spotted rose snapper (*L. guttatusclarkia*) showed that fishmeal can be replaced with 35% of meat and meat bone meal and tuna meal by product without compromising growth (Hernandez et al. 2016). Hu et al. (2008) showed that 67% fishmeal can be replaced with a combination of poultry by-product meal and meat and bone meal in the diet of gibel carp, (*C. auratus gibelio*). However, a study by Chaklader et al. (2020) showed that full replacement of fishmeal with poultry by product supplemented with methionine negatively affect growth, liver health and histological characteristics of different organs of juvenile barramundi, *L. calcarifer*. Animal protein sources may have a high lipid or ash content which results in reduced digestibility of the feed by fish (Kureshy et al. 2000).

Studies have indicated that a combination of plant and animal protein sources can replace a large proportion of fishmeal in the diet of carnivorous species (Koch 2016, Cabano 2017). A study by Seo et al. (2022) on carnivorous olive flounder (*P. olivaceus*) showed that fishmeal replacement of up to 35% with a combination of poultry by product meal, tuna by product meal, soybean meal, black soldier larvae meal and black soldier fly oil can be achieved without negatively affecting fish growth. Similarly, a study on juvenile dusky kob (*A. japonicus*) by Jones et al. (2021) found that length gain can be improved by replacing 50% of dietary fishmeal with a favourable balance of meals processed from poultry, blood, canola and soybean supplemented with crystalline amino acid.

The combination of animal and plant protein sources in the diet of cultured species have a potential to increase profitability by reducing the cost of diet and potentially reducing the

nutrient content in effluent waste (Sullivan 2008). ** It is, therefore, important to investigate the effect of fishmeal replacement with alternative protein sources (chicken meal, blood meal, and soya in equal proportions) on the culture of juvenile yellowtail, (*S. lalandin*) in a RAS. The aim of this study was to determine the maximum replacement level of fishmeal with alternative protein sources (chicken meal, blood meal, and soya in equal proportions) in the diet of juvenile yellowtail (*S. lalandin*), which optimised survival, growth, FCR, and fish heath in a RAS.

The objectives of the study were to assess the (i) survival, (ii) growth, (iii) feed conversion ratio and (iv) fish health of juvenile yellowtail fed formulated feeds with levels of fishmeal replacement by alternative and more sustainable protein sources.

Materials and methods

Experimental animals

Juvenile yellowtail (*S. lalandi*) spawned and reared by Kingfish Enterprises (Pty) Ltd were donated to the project. Fish were initially fed with a commercial yellowtail diet (Aqua Management Technologies (AMT)) is currently being used at the Kingfish Enterprises. The AMT diet consisted of 47.6% protein, 10.6% lipid, 17.3% carbohydrates and 13.07 Mg/kg energy.

Prior to the start of the experiment, fish were starved for 24 hours. At the beginning of the experiment, fish were anaesthetised using 0.2 mL/L 2-phenoxyethanol and once sedated were individually weighed (g) and measured (mm). A total of 300 juvenile yellowtail were randomly assigned into twelve tanks with 25 fish per tank.

For the experiment, fish were fed to satiation two times a day, satiation was assumed when fish stopped feeding at the surface. Individual containers of food were assigned to each tank and were weighed at the start of the experiment and after each week of feeding.

Experimental system

The experimental RAS described in Chapter 2 was used for this experiment.

Water temperature (°C), dissolved oxygen (mg/L), pH and total ammonia nitrogen (TAN) were measured daily while nitrate (mg/L) was measured once a week. The toxic ammonia (NH_3) portion was calculated using TAN, Temperature and pH.

Dietary treatments

Four experimental diets were formulated to be iso-nitrogenous (44% protein) and iso-calorific (19.50 Mg/Kg) as follows: a control diet with 100% fishmeal as the only protein source (which was the same formulation as the 44% diet used in the first experiment), (2) control diet in which 33% of 67% fishmeal was replaced with the protein mixture, (3) control diet in which 67% of 33% fishmeal was replaced with the protein mixture and (4) control diet in which 100% of fishmeal was replaced with the protein mixture (Table 4.1). The experimental diets were formulated with the assistance of Professor Cliff Jones (Rhodes University) and manufactured by Marifeed (Pty) Ltd. in Hermanus, South Africa. Marine fish oil (Oceana (Pty) Ltd, Cape Town, South Africa) was used as the lipid source whereas vegetable starch was used as the source of carbohydrates.

Preparatory technologies developed for the abalone-farming industry were used to extrude feed through a circular 4.0 mm die and pellets were then cut at 3 mm long. The pellets were then dried at 38 °C for 18 hours, cooled to room temperature and packaged.

The experimental diets were randomly assigned to three replicate tanks for the 49 days of the experiment.

Table 4.1. Formulation and proximate composition of the experimental diets (g/10g dry weight) used to feed juvenile yellowtail, *S. lalandi* in a RAS.

Diets (% fishmeal)	**100%**	**67%**	**33%**	**0%**
Ingredients				
Fishmeal	6.38	4.45	2.19	0.00
Soya	0.00	0.94	2.04	3.11
Starch	2.54	2.12	1.62	1.15
Blood meal	0.00	0.37	0.81	1.24
Poultry meal	0.00	0.70	1.51	2.30
Canola meal {rapeseed)	0.00	0.52	1.13	1.72
Vitamix	0.01	0.01	0.01	0.01
Marine Fish Oil	1.10	0.89	0.68	0.48
Total (dry weight) (g/10g)	**10.00**	**10.00**	**10.00**	**10.00**
Protein (%)	44.00	44.00	44.00	44.00
Lipid** (%)	17.19%	15.82%	14.21%	12.66%
Energy** (MJ/kg)	19.50	19.50	19.50	19.50
Protein** (g): Energy (MJ/kg)	2.26	2.26	2.26	2.26
Methionine (%, g meth. /100 g diet)	1.22	0.92	0.56	0.21
Methionine (%, g meth. /100 g protein)	2.77	2.08	1.27	0.48
Lysine (%, g lysine/100 g diet)	3.22	2.51	1.68	0.88
Lysine (%, g lysine/100 g protein)	7.32	5.71	3.53	1.99
Analyzed Composition				
Crude protein (% dry matter)	45.21±0.21	46.39±0.75	47.32±0.74	48.55±0.28
Crude lipid (%)	16.03±0.24	13.78±0.3	12±0.90	10.38±0.65
Ash (%)	14.63±0.16	12.27±0.41	9.87±0.86	7.01±0.33
Moisture	6.12±0.17	7.13±0.36	7.78±0.17	7.69±0.10

**Calculated

The experiment ran for total of 49 days with fish being sedated, weighed (g) and measured (mm) on day 0, 21 and 49. At the end of the experiment, four fish from each tank were randomly selected and killed by over sedation with 2-phenoxyethanol followed by pithing. Two fish were used for liver histology and two for proximate composition. The remaining fish were returned to Kingfish Enterprises who incorporated them back into the farm's production.

Proximate analysis

Fish were cut up and dried in an ExoTherm digital oven for 24 hours at 70°C. After drying, fish were then ground by pestle and mortar. Proximate composition of fish and feed were then analysed by the Soil Fertility and Analytical Services at KwaZulu Natal Department of Agriculture and Rural Development. The methods for proximate analysis are outlined in Chapter 2.

Histological preparation and analysis

The two fish used to determine HIS had samples of their liver which were fixed in 10% buffered formalin for histological examination. Liver samples were then sent to African Aquatic Vet Services (Pretoria) for preparation of slides Fractions of each sampled liver was dehydrated in a graded ethanol series and then embedded in paraffin wax. Haematoxylin and Eosin (H&E), periodic acid-Schiff diastase (PASD) and periodic acid-Schiff (PAS) were used to stain four µm sections of the liver for light microscopy examination (Genten et al. 2009).

Examinations of slides were performed using a BM2000 Microscope at magnification of 40X. Hepatocyte nuclei were considered normal if the shape was spherical, with a central nucleoli

and abnormal if the nucleus was shrunken and nucleoli was not central. One hundred Lipid vacuole zones were measured on each liver section.

Statistical analysis

Percentage weight gain, specific growth rate (SGR), food conversion ratio (FCR), condition factor (CF), protein efficiency ratio (PER) and hepatosomatic index (HIS) were all calculated using the following formulae.

$$\% \text{ Weight gain} = [(\text{final weight (g)} - \text{initial weight(g)}) \div \text{initial weight(g)}] \times 100$$

$$\text{SGR} = [\text{Ln (final weight(g))} - \text{Ln (initial weight(g))}] \div 100 / \text{time (days)}$$

$$\text{CF} = 100 \times \text{whole body weight (g)} \div (\text{body length [cm]})^{2.84}$$

$$\text{FCR} = \text{dry feed supplied(g)} \div \text{wet weight gained (g)}$$

$$\text{PER} = \text{body weight gain (wet weight g)} \div \text{protein ingested (g)}$$

$$\text{HSI} = 100 \times (\text{liver weight [g]}) \div (\text{body weight [g]})$$

Normality of the data was checked using a Kolmogorov-Smirnov test while the homogeneity of variances was checked using Levene's Test. A One-way ANOVA was used to compare dietary treatments. Tukey's multiple range test was used to identify differences between individual diets. Significance was set at $p < 0.05$.

Results

Water quality

Total ammonia nitrogen (TAN), Daily unionized ammonia (NH_3), temperature, nitrate, pH, dissolved oxygen and percentage saturation of dissolved oxygen in experimental RAS are represented in Table 4.3.

Table 4.3: Water quality parameters maintained for the entire duration of the study.

Parameter	**Range**
Temperature (° C)	21.57±0.88
Dissolved oxygen (mg/L)	6.3±0.23
Dissolved oxygen (% saturation)	80.7±11.4
Total ammonia nitrogen (TAN)	0.84±0.23
pH	7.48±0.08
NH_3 (mg/L)	0.004±0.01
Nitrate (mg/L)	7.7±1.31

Survival

Survival was significantly greater for fish fed 33% and 100% fishmeal (One-Way ANOVA, $F = 16.68$ $df = 3$, $p = 0.003$) compared to fish fed 0% fishmeal diet. There were however no differences in survival between fish fed 33, 67, and 100% fishmeal (Figure 4.1).

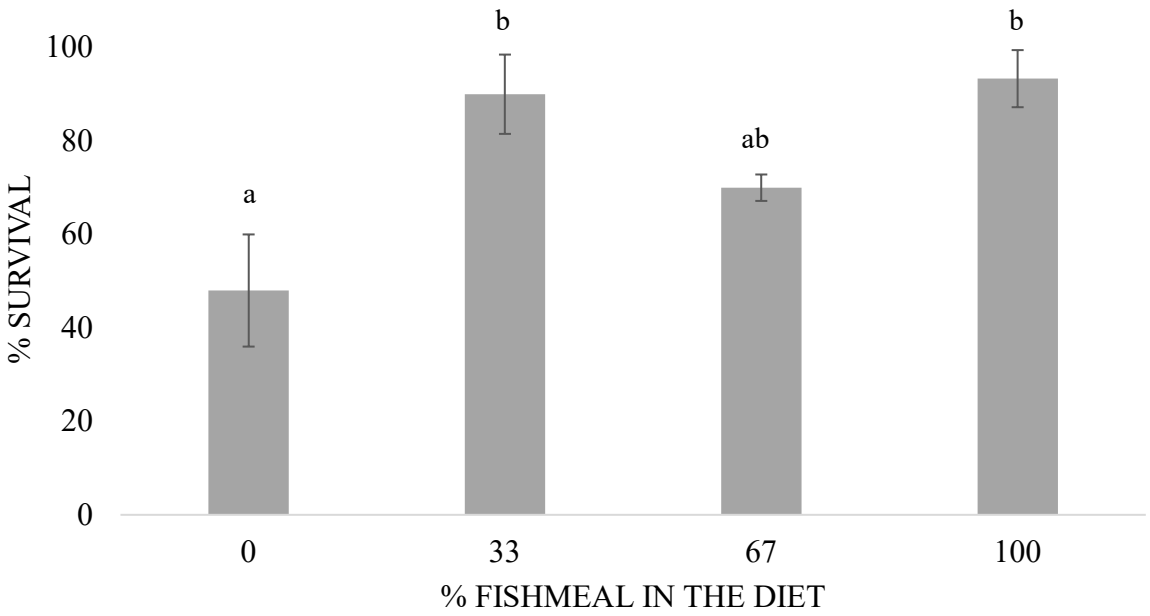

Figure 4.1: Percentage Survival of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being fed a control diet with 100% fishmeal as the only protein source, 67% fishmeal, 33% fishmeal and 0% fishmeal with terrestrial protein sources. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

Growth performance

Overall, SGR was greater for fish fed 67 and 100% fishmeal (One-Way ANOVA, $F = 6.27$ *df* $= 3$, $p = 0.017$) compared to fish fed 0% fishmeal. There were however no differences in SGR between fish fed 33, 67, and 100% fishmeal (Figure 4.2).

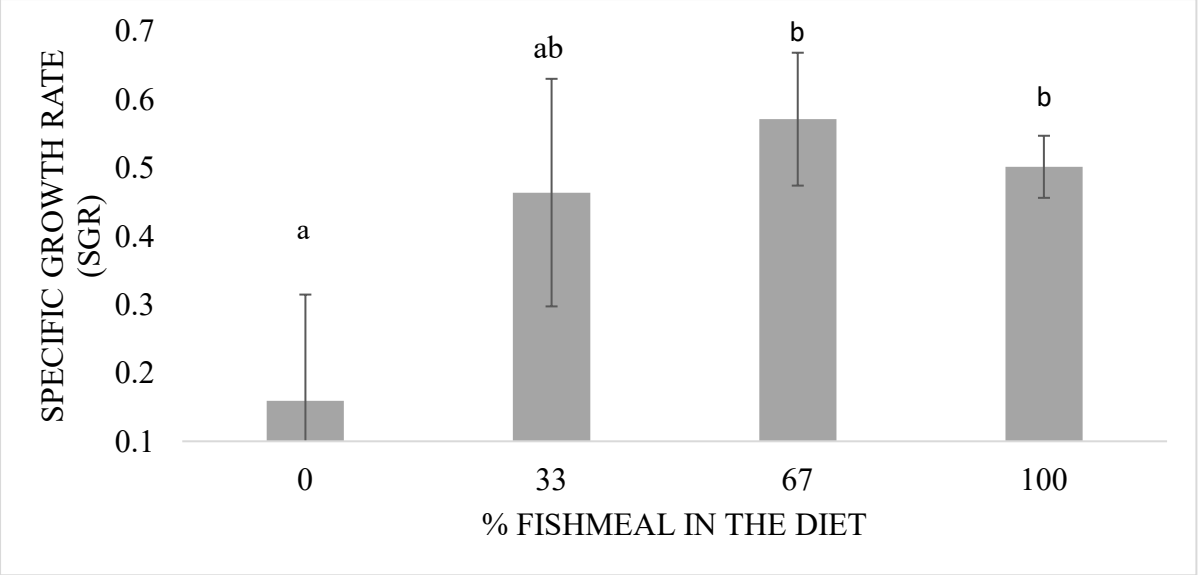

Figure 4.2: Specific Growth Rate (SGR) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being fed a control diet with 100% fishmeal as the only protein source, 67% fishmeal, 33% fishmeal and 0% fishmeal with terrestrial protein sources. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

Average weight was significantly affected by dietary treatments and time with differences in length from day 14 onwards. On day 14 (One-Way ANOVA, $F = 23.42$ $df = 3$, $p< 0.001$). and 28 (One-Way ANOVA, $F = 17.2$ $df = 3$, $p = 0.001$), average weight was significantly higher for fish fed 33%, 67% and 100% fishmeal diet compared to fish fed 0% fishmeal diet. On the last day of the experiment, average weight was greater for fish fed 67% and 100% fishmeal diet compared to fish fed 0% fishmeal diet (One-Way ANOVA, $F= 5.77$ $df=3$, $p= 0.02$ but no differences were found between fish fed 33%, 67% and 100% fishmeal diet (Figure 4.3).

Average length was greater for fish fed 33%, 67% and 100% fishmeal diets compared to fish fed 0% fishmeal on day 28 (One-Way ANOVA, $F= 6.17$ $df=3$, $p= 0.02$). There were however no differences in average length between all dietary treaments on day 49 (Figure 4.3).

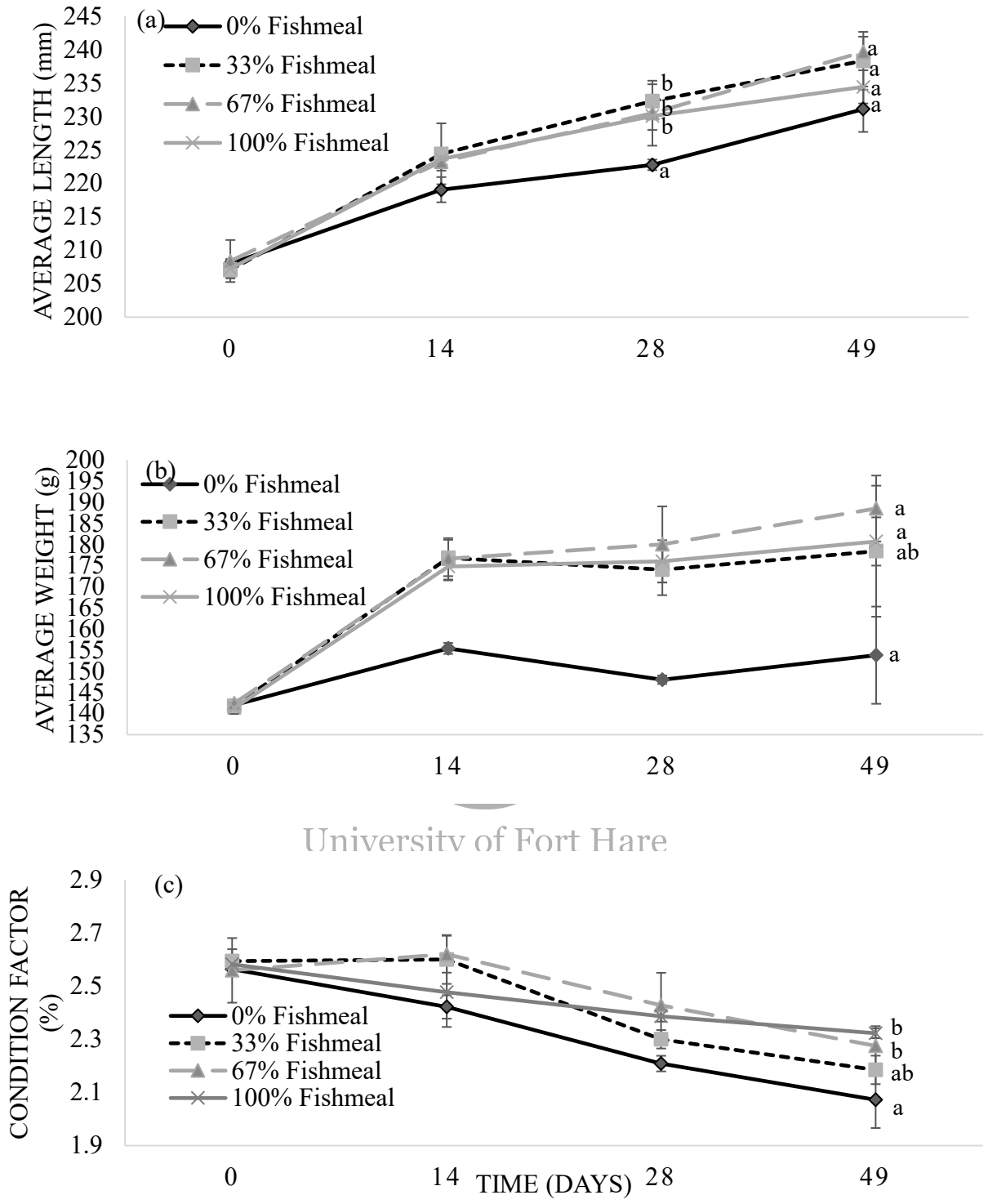

Figure 4.3: The mean length (a), mean weight (b) and CF (c) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being a control diet with 100% fishmeal as the only protein source, 67% fishmeal, 33% fishmeal and 0% fishmeal with terrestrial protein sources. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

The changes in CF were significantly affected by the interaction between dietary treatment and time with average values lower at the end of experiment compared to the start of the experiment. On day 28 (One-Way ANOVA, F= 6.49 df=3, p= 0.02) and 49 (One-Way ANOVA, F= 7.31 df=3, p= 0.01), CF of fish fed 67% and 100% fishmeal diet differed significantly from those fed the 0% fishmeal diet but 33%, 67% and 100% fishmeal diets did not differ from each other (Figure 4.3)

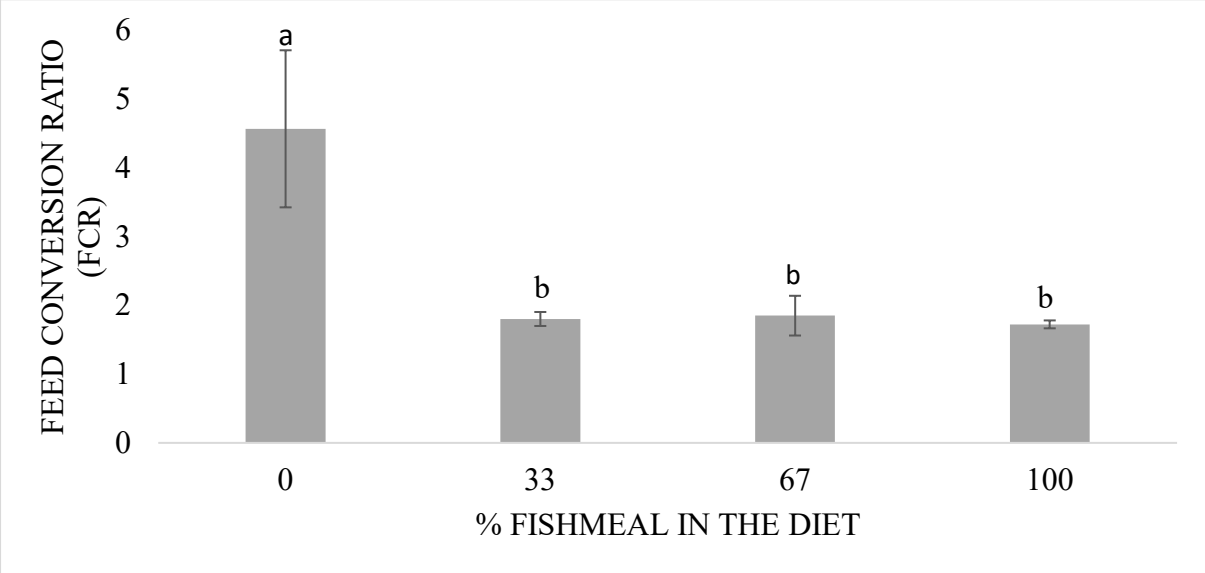

Figure 4.4: Feed Conversion Ratio (FCR) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being a control diet with 100% fishmeal as the only protein source, 67% fishmeal, 33% fishmeal and 0% fishmeal with terrestrial protein sources. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

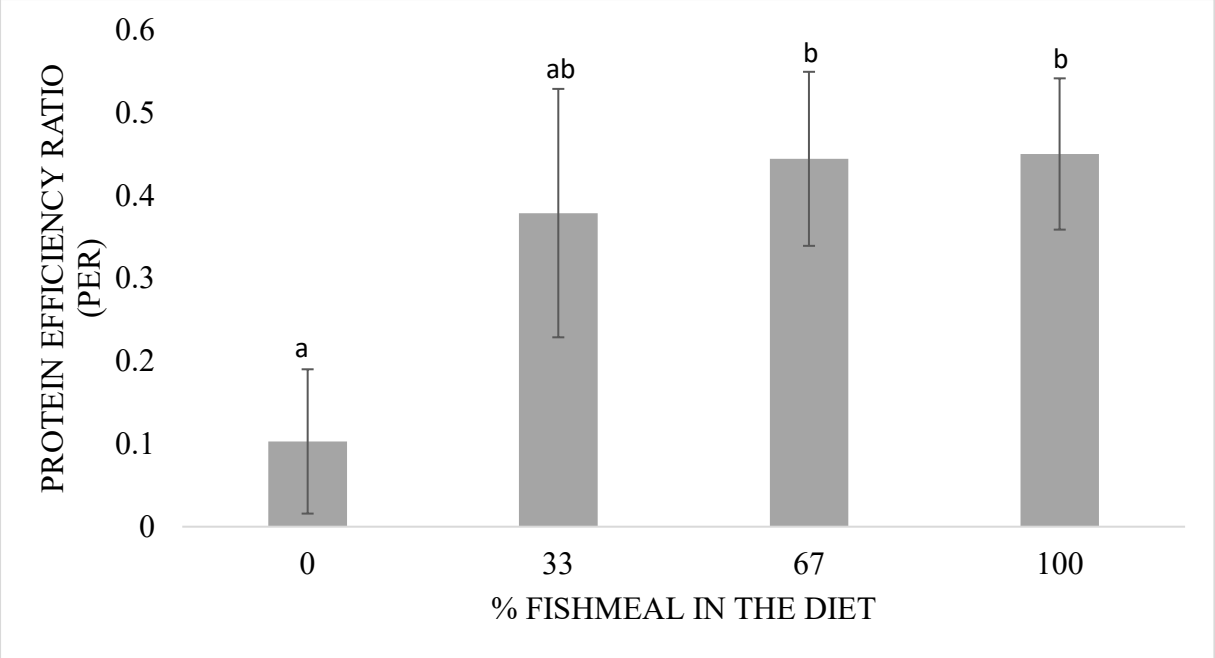

Figure 4.5: Protein Efficiency Ratio (PER) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being fed a control diet with 100% fishmeal as the only protein source, 67% fishmeal, 33% fishmeal and 0% fishmeal with terrestrial protein sources. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

Feed efficiency

Feed conversion ratio (FCR) was significantly affected by fishmeal replacement with diets having fishmeal having lower FCRs compared to the diet with no fishmeal at all (One-Way ANOVA, F = 16.09 *df*=3, p= 0.001) (Figure 4.5).

Fish fed diets having 67% and 100% fishmeal had high PER (One-Way ANOVA, F=5.36 *df*=3, p= 0.026)compared to fish fed 0% fishmeal. There were however no differences in PER between fish fed 33%, 67% and 100% fishmeal (Figure 4.5).

Table 4.3: The effect of fishmeal replacement on survival, SGR, FCR, PER and CF of Juvenile yellowtail, *Seriola lalandi* fed after 49 days. Data are mean values ± standard deviation of three replicates.

Diets (% fishmeal)	**0%**	**33%**	**67%**	**100%**
Parameter				
Survival (%)	48.00±25^{a}	90.00±8.49^{b}	70.00±2.83^{ab}	93.33±6.11^{b}
SGR	0.16±0.16^{a}	0.46±0.17^{ab}	0.54±0.10^{b}	0.50±0.05^{b}
FCR	4.6±1.15^{a}	1.8±0.10^{b}	1.8±0.32^{b}	1.7±0.06^{b}
PER	0.11±0.10^{a}	0.38±0.15^{ab}	0.44±0.11^{b}	0.45±0.11^{b}
FCR	4.6±1.15^{a}	1.8±0.10^{b}	1.8±0.32^{b}	1.7±0.06^{b}
CF	2.07±0.11^{a}	2.19±0.05^{ab}	2.28±0.07^{b}	2.32±0.09
HSI	0.89±0.10^{a}	0.83±0.10^{a}	0.80±0.07^{a}	0.87±0.13^{a}

Values in each row with different superscripts indicates significant differences ($p < 0.05$).

Microscopic evaluation of the liver and hepatosomatic index

The hepatocytes of fish fed fishmeal diets showed large lipid vacuole zones in the cytoplasm (One-Way ANOVA, $F=7.57$ $df=3$, $p<0.001$) compared to the 0% fishmeal. These treatments also showed spherical nuclei located in the centre of the cells which is an indication of healthy liver (Figure 4.7).

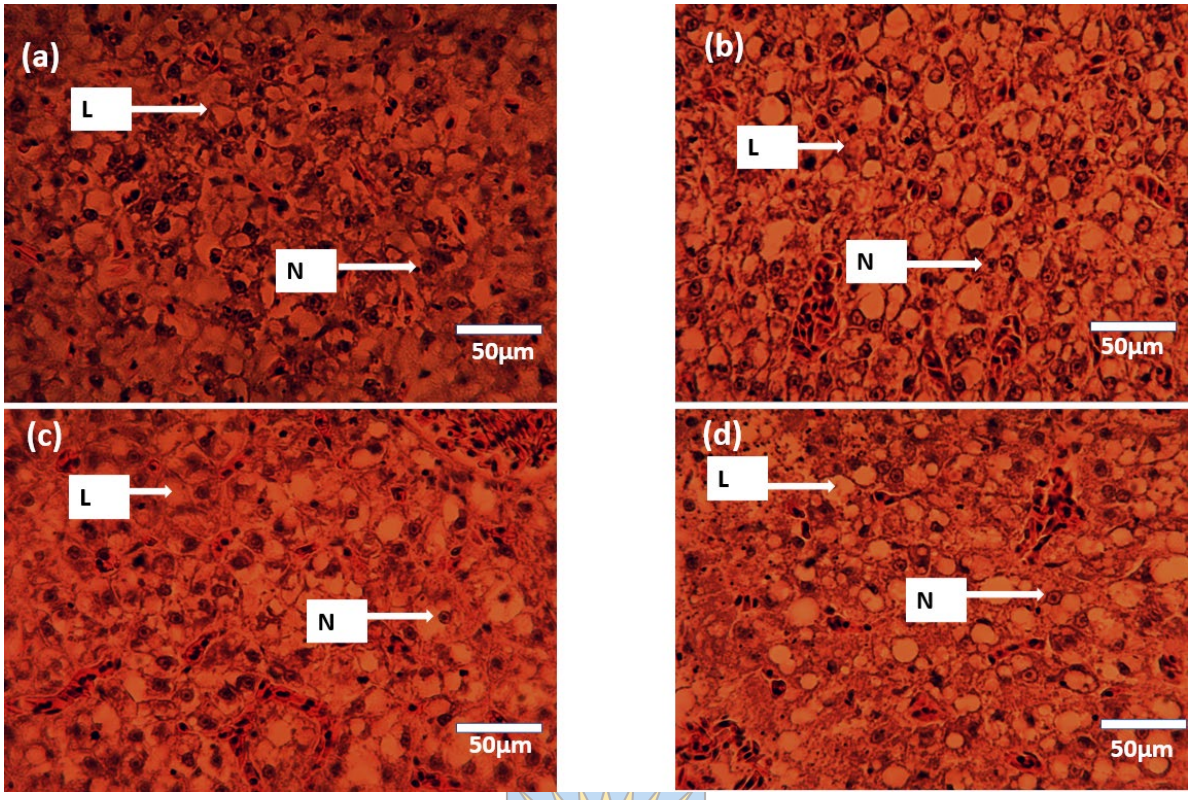

Figure 4.7: Liver section (400x) of *Seriola lalandi* after 49 days of being fed a control diet with (a) 0% fishmeal, (b) 33% fishmeal, (c) 33% fishmeal and a (d) 100% fishmeal. L represents lipid vacuoles while N represent nuclei.

Hepatosomatic index was not affected by levels of fishmeal replacement in the diet (Figure 4.8).

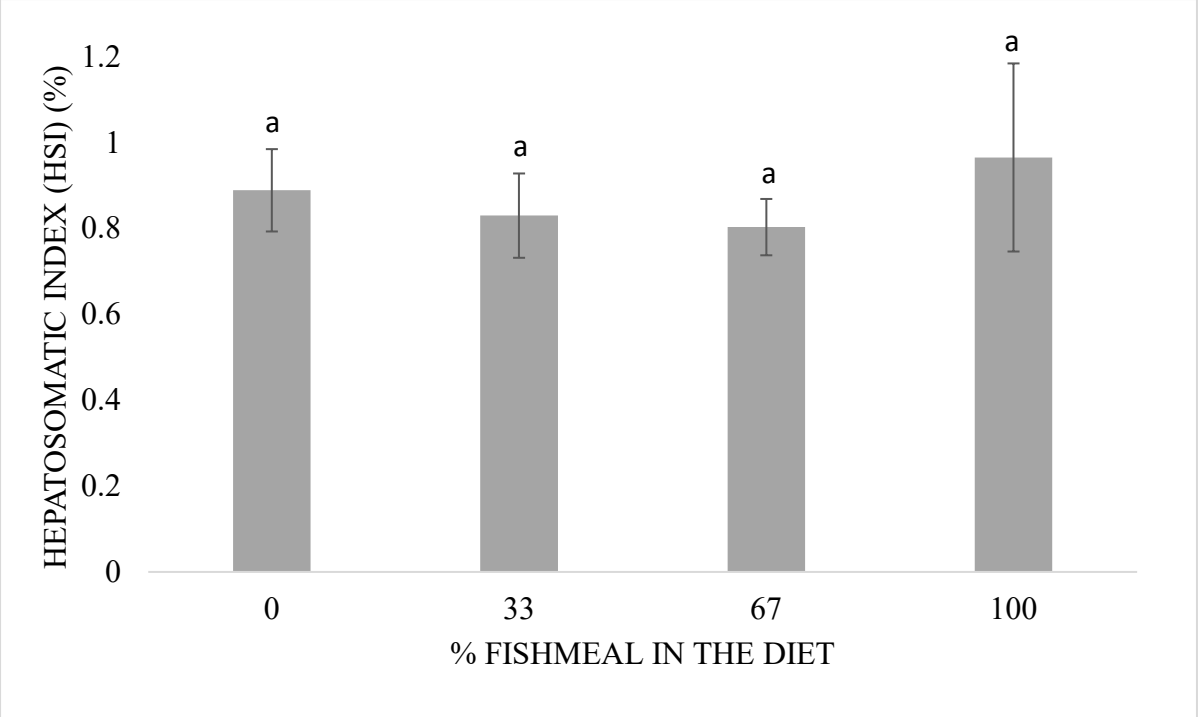

Figure 4.8: Hepatosomatic index (HSI) of juvenile yellowtail kingfish, *Seriola lalandi* after 49 days of being fed a control diet with 100% fishmeal as the only protein source, 67% fishmeal, 33% fishmeal and 0% fishmeal with terrestrial protein sources. Error bars denote standard deviations of the mean. Different superscript letters indicate significant differences ($p<0.05$).

Proximate composition analysis

Body protein, ash, moisture and lipid was not affected by partial and full replacement of fishmeal (Table 4.4).

Table 4.4: Proximate composition of whole body of juvenile yellowtail, *Seriola lalandi* after 49 days of being fed levels of fishmeal replacement. Data are mean values ± standard deviation of three replicates.

Diets (% fishmeal)	**0%**	**33%**	**67%**	**100%**
Proximate composition				
Moisture (%)	11.76±9.40[a]	7.59±1.42[a]	5.92±3.30[a]	7.32±3.63[a]
Crude protein (%)	61.63±1.61[a]	65.48±3.86[a]	66.68±6.76[a]	67.76±4.04[a]
Crude lipid (%)	19.4± 4.84[a]	20.9±0.30[a]	16.67±4.72[a]	5.92±3.30[a]

Ash (%)	12.14±1.04[a]	14.46±1.81[a]	13.69±2.54[a]	17.28±3.83[a]

Values in each row with different superscripts indicates significant differences ($p < 0.05$).

Discussion

Formulated diets should either improve or maintain survival for it to be accepted. Survival was significantly higher for fish fed fishmeal diets compared to the 0% contradicting previous studies on other carnivorous species (Hu et al. 2008, Glencross et al. 2016). During the experiment it was observed that fish fed the 0% fishmeal diet were not as active as the fish fed fishmeal diets in terms of swimming and feeding. The health of the fish may have been impaired at the 0% fishmeal diet hence the low survival rate. Savonitto et al. (2021) reported that survival on *S. aurata* could have been resulted from stress of the anaesthtic which could also be the case for this study.

The partial replacement of fishmeal has been met with varied success in carnivorous finfish species (Hernandez et al. 2016, Moutinho et al. 2017). In this study, diets with 33% and 67% fishmeal with a blend of soybean meal, blood meal, poultry meal and canola meal produced equivalent growth as the 100% fishmeal diet while the diet with no fishmeal produced the worst growth. In a study by Jones et al. (2021), a replacement of 50% fishmeal with a balance of soybean meal, poultry meal, canola meal and blood meal in the diet of dusky kob (*A. japonicus*) improved growth whereas a diet with no fishmeal had the lowest growth. The results from this study and Jones et al. (2021) suggests that the fish fed diets with no fishmeal could not adequately use the dietary protein as those fish fed diets with fishmeal and that full replacement of fishmeal with the combination of soya, blood meal, poultry meal and canola meal results in growth detoriation. Full replacement of fishmeal with poultry by product meal supplemented

with 0.40% methionine in the diet of *L. calcarifer* also resulted in poor growth (Chaklader at al. 2020). The reason could be that the diets with no fishmeal were not nutritionally balanced.

Although differences were found in the CF of fish fed fishmeal diets compared to 0% fishmeal diets, the CF was lower across all dietary treatments at the end of experiment. Several authors have suggested that a drop in CF is a result of stress (Goede 1990, Jones et al 2021). This suggests that the fish in all dietary treatments of this study were stressed towards the end of the experimenthowever this could mean that lower CF was a result of stress instead of the diets tested..

Studies have shown that full replacement of fishmeal with alternative protein sources have resulted in negative impact on the FCR (Yigit 2006, Glencross 2016, Chaklader 2020). Feed conversion ratio was significantly higher for fish fed the diet with no fishmeal (4.6) compared to all fishmeal inclusion diets (1.7-1.8) in the present study. Similar results have been found for juvenile *L. calcarifer* (Chaklader at al. 2020), *A. japonicus* (Jones et al. 2021). The high FCR found on 0% fishmeal diet suggests that full replacement of fishmeal with the blend of soya, blood meal, poultry meal and canola meal can result from increased antinutritional factors or a lack in essential amino acids and therefore growth of the fish was negatively affected (Lall and Anderson 2005, Xu and Hanna, 2011).

Protein efficiency ratio (PER) is mostly used in nutrition studies to determine how efficiently protein supports growth (Rossetti 2012). The PER was higher for fish fed fishmeal diets compared to fish fed the 0% fishmeal diet. The low PER in fish fed the 0% fishmeal diet may suggest that a proportion of protein was used for other purposes such as energetic requirements, deaminated and excreted as ammonia or deposited as fat instead of growth (Daniel 2004). The high PER in fish fed fishmeal inclusion diets suggests that the dietary protein was efficiently used to support growth.

Assessing the health status of the fish give in nutrition studies is important because it determines the acceptability of formulated feed by fish (Raskovic et al 2011). Fish fed fishmeal diets showed high levels of lipid vacuolated zones compared to the 0% fishmeal diet. High levels of lipid deposition in the liver represents a well-fed status (Tacon et al. 1997). This suggests that the 0% fishmeal diet could not meet dietary requirements of the fish hence the low survival in fish fed the 0% fishmeal diet. Histological changes in the hepatocyte nuclei are a result of inadequate feed. There were however no histological changes observed across all dietary treatments. The changes could however occur in a long term.

Hepatosomatic index (HSI) is an important parameter to assess in fish nutrition studies as it provides an indication of the nutrition condition of fish (Brusle' and Anadon 1996). The average HSI values in this experiment were similar across all dietary treatments. They were however lower (0.8-0.89) than those of other carnivorous species. Higher HSI values were found for dusky kob, *A. japonicus* (0.84-1.80 and 1.8-2.9) by Rossetti (2012) and Jones et al. (2021) respectively and were 1.54-1.89 for gilthead seabream, *S. aurata* (Cabano 2017). The high HSI values may be associated with high dietary starch levels (Jones et al. 2021) and essential amino acid deficiency in the diet (Castell et al. 1972). The relatively low levels of starch of the diets use in this experiment might have had the impact on the low HSI values. The values found in this experiment could also mean that the fish were able to successfully utilize lipid for energetic requirements (Rossetti 2012).

The whole-body proximate composition of a fish helps to provide a general indication of its nutritional status (Marais, 1990). In this experiment, whole body protein, lipid, ash and moisture of *S. lalandi* were not affected by different fishmeal levels in the diet. Similar results were reported for gibel carp (*C. auratus gibelio*) fed a combination of meat and bone meal and poultry by-product meal, alone or supplemented with methionine and lysine (Hu et al. 2008) and juvenile black sea bass (*C. striata*) (Sullivank 2008) fed a combination of meat and bone

meal and poultry by-product meal. These results indicate that a high proportion of fishmeal in the diet of *S. lalandi* can be replaced by a combination of soya, blood meal, poultry meal and canola meal without having an impact on whole body proximate composition.

The results indicate that full replacement of fishmeal (0% diet) had the worst SGR, FCR & PER. The remaining three diets were statistically similar which suggests that a diet with 33% fishmeal produces as good growth, FCR and PER as 67 and 100% fishmeal diets. However, as there was also similarity in SGR and PER for the 0 and 33% diets, it is therefore recommended that future experiments focus on the region between 33– 67% to find the optimal break point for the various growth and feeding indicators.

CHAPTER 5

GENERAL DISCUSSION

In this final discussion the relevant literature and results from chapter 3 and 4 are summarized in terms of their contribution towards optimization of dietary protein and fishmeal substitution for juvenile yellowtail while comparing them to other carnivorous marine species. Future potential studies are also suggested.

Survival, SGR, FCR and PER from the two experiments are summarized in table 5.1

The population of the world and man's demand for seafood is increasing (Msangi et al. 2013). Aquaculture will play a major role in improving nutrition and food security. Aquaculture having already surpassed the total annual volume of seafood harvested for human consumption (Sofia 2022). Yellowtail kingfish, *Seriola lalandi* is one of the marine finfish species farmed around the world in cages (Stephens and Savage 2010). Several diets have been formulated for aquaculture of *S. lalandi* in cages, but limited formulations have been specifically for RAS and tested experimentally.

Seriola lalandi, like other carnivorous fish require high protein levels in their diet for their optimum growth (Moran et al. 2010, Stuart et al. 2018). Fishmeal has been used as the main protein source when formulating carnivorous fish feeds but with the growth of aquaculture sector it is becoming more expensive and increasingly unsustainable (Webster et al. 1992, Francis et al. 2001, Bowyer et al. 2013). Fish farmers are therefore constantly trying to reduce .the amount used or replace it with suitable alternative protein sources (Jirsa et al. 2011, Bowyer et al. 2013, Buentello et al. 2015, Herman and Schmidt 2016). This study therefore aimed to

contribute to the optimization of dietary protein and fishmeal substitution in the diet of juvenile yellowtail, *S. lalandi* in a Recirculating Aquaculture Systems (RAS).

Table 5.1: Survival, growth and feed related parameters from the growth trials outlined in Chapter 3 and 4.

Experiment (Chapter no.)	Optimal condition	%Survival	SGR	FCR	PER
Crude protein (3)	44% protein	100	2.98±0.05	1.42±0.05	1.6±0.05
Fishmeal replacement (4)	33% fishmeal	63.87±33.70	0.46±0.17	1.8±0.10	0.38±0.15

Optimal condition= best growth at lowest cost, SGR=Specific Growth Rate, FCR= Feed Conversion Ratio, PER= Protein Efficiency Ratio

One of the major costs affecting the aquaculture industry is the high price of feed which constitutes about 50% of the total operating costs (Woolley 2009, Trushenski et al. 2011, Craig and Helfrich 2017). In order for a fish farm to succeed, formulated feeds should produce good survival, growth, FCR and fish health at lowest possible cost. Protein is the main ingredient when formulating diet for carnivorous species and is the most expensive component. In an effort to reduce the cost of the diet, it is necessary to focus on optimizing the dietary protein requirement of carnivorous species. Chapter 3 investigated the effect of dietary protein level on survival, growth, FCR and health of juvenile yellowtail in a RAS. The results showed that a dietary protein inclusion level of 44% fishmeal produced good growth and health similar to the 50 and 56% fishmeal protein diets. In terms of producing the best growth at the lowest FCR, the 44% protein diet was found to be optimal for juvenile yellowtail in a RAS.

The 44% optimal dietary protein level for juvenile yellowtail found in this study is lower than dietary protein requirement found for other carnivorous species. The higher protein requirements were found for juvenile yellowtail flounder, *L. ferruginea* (55%) (Dwyer et al. 2002), golden mandarin fish, *S. scherzeri* (55%) (Sankian 2017) and dusky kob, *A. japonicus* (45%) (Daniel 2004). These results can be beneficial to fish farmers as low dietary protein level has a potential to reduce the cost of farming the species. Further research into dietary protein requirement of different size classes of yellowtail kingfish is needed to fully optimise diet at different stages of grow out.

Chapter 3 determined the optimal dietary protein level for the culture of juvenile yellowtail in a RAS with fishmeal being used as the sole dietary protein source. In Chapter 4, different levels of fishmeal were replaced with a blend of soya, blood meal, poultry meal and canola meal in iso-nitrogenous (44% protein) and iso-calorific (19.50 Mg/Kg) diets. The results showed that fishmeal replacement of up to 67% can be replaced with a blend of soya, blood meal, poultry meal and canola meal without having a negative impact on survival, growth and FCR. However, 33% fishmeal diet (67% alternative protein sources) was statistical similar to the 0% fishmeal diet (100% alternative protein sources) that produced the worst growth, FCR and PER in terms of SGR and PER. It is therefore recommended that future studies should focus on finding the optimal breakpoint for different growth and feeding indicators in the region between 33% and 67% fishmeal inclusion. Since the fishmeal replacement of up to 67% did not improve growth it is also recommended that future studies should also focus on substituting fishmeal with other less expensive and sustainable protein sources which might improve growth.

Based on the cost and amino acid profile, canola meal is cheaper and has a better amino acid profile than soybean meal (Sajjadi and Carter 2004), so future studies should also test the same formulations as this study but removing soybean meal from the formulation.

REFERENCES

Asche, F. and Tveterås, S., 2004. On the relationship between aquaculture and reduction fisheries. *Journal of Agricultural Economics*, *55*(2), pp.245-265.

Besson, M., Komen, H., Rose, G. and Vandeputte, M., 2020. The genetic correlation between feed conversion ratio and growth rate affects the design of a breeding program for more sustainable fish production. *Genetics Selection Evolution*, *52*, pp.1-10.

Booth, M.A., Allan, G.L. and Pirozzi, I., 2010. Estimation of digestible protein and energy requirements of yellowtail kingfish Seriola lalandi using a factorial approach. *Aquaculture*, *307*(3-4), pp.247-259.

Bowen, S.H., 1979. A nutritional constraint in detritivory by fishes: the stunted population of Sarotherodon mossambicus in Lake Sibaya, South Africa. *Ecological monographs*, *49*(1), pp.17-31.

Bowyer, J.N., Qin, J.G., Smullen, R.P. and Stone, D.A.J., 2012. Replacement of fish oil by poultry oil and canola oil in yellowtail kingfish (Seriola lalandi) at optimal and suboptimal temperatures. *Aquaculture*, 356, pp.211-222.

Bowyer, J. N., Qin, J. G., and Stone, D. A. (2013). Protein, lipid and energy requirements of cultured marine fish in cold, temperate and warm water. *Reviews in Aquaculture*, *5*(1), 10-32.

Bregnballe, J., 2010. A guide to recirculation aquaculture: an introduction to the new environmentally friendly and highly productive closed fish farming systems.

Brunty, J.L., Bucklin, R.A., Davis, J., Baird, C.D. and Nordstedt, R.A., 1997. The influence of feed protein intake on tilapia ammonia production. *Aquacultural engineering*, *16*(3), pp.161-166.

Brusle, J. and Anadon, G.G., 1996. The structure and function of fish liver Fish Morphology. *Edited by Munshi, JSD and Dutta, HM Science Publishers Inc*.

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

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

Bureau, D.P., Harris, A.M. and Cho, C.Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (Oncorhynchus mykiss). *Aquaculture*, *180*(3-4), pp.345-358.

Cabano, M.O., 2017. *Impact of fish meal replacement by alternative and sustainable ingredients in diets for gilthead seabream (Sparus aurata Linnaeus, 1758) juveniles* (Doctoral dissertation, Universidade do Algarve (Portugal)).

Cabral, E.M., Bacelar, M., Batista, S., Castro-Cunha, M., Ozório, R.O.A. and Valente, L.M.P., 2011. Replacement of fishmeal by increasing levels of plant protein blends in diets for Senegalese sole (Solea senegalensis) juveniles. *Aquaculture*, *322*, pp.74-81.

Castell, J.D., Sinnhuber, R.O., Wales, J.H. and Lee, D.J., 1972. Essential fatty acids in the diet of rainbow trout (Salmo gairdneri): growth, feed conversion and some gross deficiency symptoms. *The Journal of Nutrition*, *102*(1), pp.77-85.

Catacutan, M.R. and Pagador, G.E., 2004. Partial replacement of fishmeal by defatted soybean meal in formulated diets for the mangrove red snapper, Lutjanus argentimaculatus (Forsskal 1775). *Aquaculture Research*, *35*(3), pp.299-306.

Chaklader, M.R., Siddik, M.A. and Fotedar, R., 2020. Total replacement of fishmeal with poultry by-product meal affected the growth, muscle quality, histological structure, antioxidant capacity and immune response of juvenile barramundi, Lates calcarifer. *Plos one*, *15*(11), p.e0242079.

Chen, Y., Ma, J., Huang, H. and Zhong, H., 2019. Effects of the replacement of fishmeal by soy protein concentrate on growth performance, apparent digestibility, and retention of protein and amino acid in juvenile pearl gentian grouper. *Plos one*, *14*(12), p.e0222780.

Cheng, Z.J., Hardy, R.W. and Usry, J.L., 2003. Effects of lysine supplementation in plant protein-based diets on the performance of rainbow trout (Oncorhynchus mykiss) and apparent digestibility coefficients of nutrients. *Aquaculture*, *215*(1-4), pp.255-265.

Cho, J.H., Lee, S., Lee, B.J., Hur, S.W., Kim, K.W., Son, M.H. and Yoo, D.J., 2021. A preliminary study of dietary protein requirement of juvenile marbled flounder (Pseudopleuronectes yokohamae). *Animal Nutrition*, *7*(2), pp.548-555.

Collet, P., 2007. Toward the development of a rearing protocol for juvenile dusky kob, *Argyrosomus japonicus* (Pisces: Sciaenidae). (Master's thesis). Rhodes University, Grahamstown, South Africa.

Collins, S.A., Øverland, M., Skrede, A. and Drew, M.D., 2013. Effect of plant protein sources on growth rate in salmonids: Meta-analysis of dietary inclusion of soybean, pea and canola/rapeseed meals and protein concentrates. Aquaculture, 400, pp.85-100.

Coutinho, F., Peres, H., Castro, C., Pérez-Jiménez, A., Magalhães, R., Pousão-Ferreira, P. and Oliva-Teles, A., 2016. Dietary protein requirement of zebra sea bream (D iplodus cervinus, L owe 1838) juveniles. *Aquaculture Nutrition*, *22*(2), pp.465-471.

Craig, S. R., Helfrich, L. A., Kuhn, D., and Schwarz, M. H. (2017). Understanding fish nutrition, feeds, and feeding.

Daniel, S., 2004. *Investigations into the nutritional requirements of juvenile dusky kob, Argyrosomus japonicus (Pisces: Sciaenidae), under ambient culture conditions*. (Master's thesis). Rhodes University, Grahamstown, South Africa.

Daniels, W. H., and Robinson, E. H., 1986. Protein and energy requirements of juvenile red drum (Sciaenops ocellatus). *Aquaculture*, *53*(3-4), 243-252.

De Silva, S.S. and Anderson, T.A., 1994. *Fish nutrition in aquaculture* (Vol. 1). Springer Science & Business Media.

Dunn, K., 2014. The diet, reproductive biology age and growth of yellowtail*, Seriola lalandi,* in South Africa (Master's thesis, University of Cape Town).

Dwyer, K.S., Brown, J.A., Parrish, C. and Lall, S.P., 2002. Feeding frequency affects food consumption, feeding pattern and growth of juvenile yellowtail flounder (Limanda ferruginea). *Aquaculture*, *213*(1-4), pp.279-292.

Egerton, S., Wan, A., Murphy, K., Collins, F., Ahern, G., Sugrue, I., Busca, K., Egan, F., Muller, N., Whooley, J. and McGinnity, P., 2020. Replacing fishmeal with plant protein in Atlantic salmon (Salmo salar) diets by supplementation with fish protein hydrolysate. *Scientific reports*, *10*(1), p.4194.

El-Dahhar, A. A., Rashwan, R. S., EL-Zaeem, S. Y., Shahin, S. A., Mourad, M. M., and El Basuini, M. F. (2022). Evaluation of the nutritional value of Artemia nauplii for European seabass (Dicentrarchus labrax L.) larvae. *Aquaculture and Fisheries*

El-Haroun, E.R. and Bureau, D.P., 2007. Comparison of the bioavailability of lysine in blood meals of various origins to that of L-lysine HCL for rainbow trout (Oncorhynchus mykiss). *Aquaculture*, *262*(2-4), pp.402-409.

Francis, G., Makkar, H.P. and Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, *199*(3-4), pp.197-227.

Francis, G., Makkar, H.P. and Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, *199*(3-4), pp.197-227.

Gatlin III, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R. and Overturf, K., 2007. Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture research*, *38*(6), pp.551-579.

Goede, R.W., 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. In *Am. Fish. Soc. Symp.* (Vol. 8, pp. 93-108).

Gordon, A.K., 1999. The effect of diet and age-at-waening on growth and survival of clownfish Aphiprion pecula *(Pisces: Pomacentridae).* (Master's thesis). Rhodes University, Grahamstown, South Africa.

Green, A.J., 2009. The protein and energy requirements of the South African abalone, *Haliotis midae* (Doctoral dissertation), Rhodes University, Grahamstown, South Africa.

Griffin, J.W. and Bradshaw, P.C., 2019. Effects of a high protein diet and liver disease in an in silico model of human ammonia metabolism. *Theoretical Biology and Medical Modelling*, *16*(1), pp.1-14.

Hamed, S.S., Jiddawi, N.S. and Bwathondi, P.O., 2017. Effects of blood meal as a substitute for fish meal in the culture of juvenile Silver Pompano Trachinotus blochii (Lacepède, 1801)

in a circulating aquaculture system. *Western Indian Ocean Journal of Marine Science*, *16*(1), pp.1-11.

Herman, E.M. and Schmidt, M.A., 2016. The potential for engineering enhanced functional-feed soybeans for sustainable aquaculture feed. *Frontiers in plant science*, *7*, p.440.

Hernández, C., González-Santos, A., Valverde-Romero, M., González-Rodríguez, B. and Domínguez-Jiménez, P., 2016. Partial replacement of fishmeal with meat and bone meal and tuna byproducts meal in practical diets for juvenile spotted rose snapper Lutjanus guttatus. *Latin American Journal of Aquatic Research*, *44*(1), pp.56-64.

Hernández, C., Hardy, R.W., Contreras-Rojas, D., López-Molina, B., González-Rodríguez, B. and Domínguez-Jimenez, P., 2014. Evaluation of tuna by-product meal as a protein source in feeds for juvenile spotted rose snapper L utjanus guttatus. *Aquaculture Nutrition*, *20*(6), pp.574-582.

Hertrampf, J.W. and Piedad-Pascual, F., 2003. *Handbook on ingredients for aquaculture feeds*. Springer Science & Business Media.

Hertrampf, J.W., Piedad-Pascual, F., Hertrampf, J.W. and Piedad-Pascual, F., 2000. Pulses. *Handbook on Ingredients for Aquaculture Feeds*, pp.338-350.

Hu, M., Wang, Y., Wang, Q., Zhao, M., Xiong, B., Qian, X., Zhao, Y. and Luo, Z., 2008. Replacement of fish meal by rendered animal protein ingredients with lysine and methionine supplementation to practical diets for gibel carp, Carassius auratus gibelio. *Aquaculture*, *275*(1-4), pp.260-265.

Ip, Y.K. and Chew, S.F., 2010. Ammonia production, excretion, toxicity, and defense in fish: a review. *Frontiers in physiology*, *1*, p.134.

Irish, A., 1997. *Investigations into the dietary protein requirements of juvenile dusky kob, Argyrosomus japonicus (Pisces: Sciaenidae)*. Master's thesis). Rhodes University, Grahamstown, South Africa.

Jamil, K., Abbas, G., Akhtar, R., Lin, H. and Li, Z., 2007. Effects of replacing fishmeal with animal by-products meal supplementation in diets on the growth and nutrient utilization of mangrove red snapper. Journal of Ocean University of China, 6, pp.292-298.

Jirsa, D., Davis, A., Stuart, K. and Drawbridge, M., 2011. Development of a practical soy-based diet for California yellowtail, *Seriola lalandi.* Aquaculture Nutrition, 17(4), pp.e869-e874.

Jirsa, D. O., Stuart, K. R., Salze, G. P., Rhodes, M. A., Davis, D. A., & Drawbridge, M. A. 2014. Limiting amino acids in practical diets for California yellowtail, Seriola lalandi. *Journal of the World Aquaculture Society*, *45*(6), 681-690.

Jones, C.L.W., Nel, A., Adesola, A., Shipton, T. and Kaiser, H., 2021. The effect of fishmeal replacement with terrestrial protein sources on growth, body condition and intestinal microbiota of juvenile dusky kob Argyrosomus japonicus. *African Journal of Marine Science*, *43*(1), pp.45-52.

Kaushik, S.J., 1997. Nutrition/feeding and body composition of fish. *Cahiers de Nutrition et de*.

Kobayashi, M., Msangi, S., Batka, M., Vannuccini, S., Dey, M.M. and Anderson, J.L., 2015. Fish to 2030: the role and opportunity for aquaculture. *Aquaculture economics & management*, *19*(3), pp.282-300.

Koch, J.F., Rawles, S.D., Webster, C.D., Cummins, V., Kobayashi, Y., Thompson, K.R., Gannam, A.L., Twibell, R.G. and Hyde, N.M., 2016. Optimizing fish meal-free commercial diets for Nile tilapia, Oreochromis niloticus. *Aquaculture*, *452*, pp.357-366.

Kolkovski, S., and Sakakura, Y. (2007). Yellowtail kingfish culture-Opportunities and problems. *World aquaculture*.

Kureshy, N., Davis, D.A. and Arnold, C.R., 2000. Partial replacement of fish meal with meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. *North American Journal of Aquaculture*, *62*(4), pp.266-272.

Kureshy, N., Davis, D.A. and Arnold, C.R., 2000. Partial replacement of fish meal with meat-and-bone meal, flash-dried poultry by-product meal, and enzyme-digested poultry by-product meal in practical diets for juvenile red drum. *North American Journal of Aquaculture*, *62*(4), pp.266-272.

Lall, S.P. and Anderson, S., 2005. Amino acid nutrition of salmonids: Dietary requirements and bioavailability. *Cahiers options mediterraneennes*, *63*(63), pp.73-90.

Le, K. and Fotedar, R., 2013. Dietary selenium requirement of yellowtail kingfish (Seriola lalandi). *Agricultural Sciences*, *4*(6A), pp.68-75.

Li, Y., Yang, Y., Song, L., Wang, J., Hu, Y., Yang, Q., Cheng, P. and Li, J., 2021. Effects of dietary supplementation of Lactobacillus plantarum and Bacillus subtilis on growth performance, survival, immune response, antioxidant capacity and digestive enzyme activity in olive flounder (Paralichthys olivaceus). *Aquaculture and Fisheries*, *6*(3), pp.283-288.

Magalhães, R., Guerreiro, I., Coutinho, F., Moutinho, S., Sousa, S., Delerue-Matos, C., Domingues, V.F., Olsen, R.E., Peres, H. and Oliva-Teles, A., 2020. Effect of dietary

ARA/EPA/DHA ratios on growth performance and intermediary metabolism of gilthead sea bream (Sparus aurata) juveniles. *Aquaculture*, *516*, p.734644.

Maldonado-García, M., Rodríguez-Romero, J., Reyes-Becerril, M., Álvarez-González, C.A., Civera-Cerecedo, R. and Spanopoulos, M., 2012. Effect of varying dietary protein levels on growth, feeding efficiency, and proximate composition of yellow snapper Lutjanus argentiventris (Peters, 1869). *Latin American Journal of Aquatic Research*, *40*(4), pp.1017-1025.

Marais, J.F.K., 1990. Body composition of ten marine migrants and one freshwater fish species caught in estuaries of the Eastern Cape, South Africa. *South African Journal of Marine Science*, *9*(1), pp.135-140.

Matsunari, H., Takeuchi, T., Takahashi, M. and Mushiake, K., 2005. Effect of dietary taurine supplementation on growth performance of yellowtail juveniles Seriola quinqueradiata. *Fisheries Science*, *71*, pp.1131-1135.

Mazid, M.A., Tanaka, Y., Katayama, T., Rahman, M.A., Simpson, K.L. and Chichester, C.O., 1979. Growth response of Tilapia zillii fingerlings fed isocaloric diets with variable protein levels. *Aquaculture*, *18*(2), pp.115-122.

Meng, F., Li, B., Xie, Y., Li, M. and Wang, R., 2020. Substituting fishmeal with extruded soybean meal in diets did not affect the growth performance, hepatic enzyme activities, but hypoxia tolerance of Dolly Varden (Salvelinus malma) juveniles. *Aquaculture Research*, *51*(1), pp.379-388.

Millamena, O.M., 2002. Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper Epinephelus coioides. *Aquaculture*, *204*(1-2), pp.75-84.

Mosconi-Bac, N., 1987. Hepatic disturbances induced by an artificial feed in the sea bass (Dicentrarchus labrax) during the first year of life. *Aquaculture*, *67*(1-2), pp.93-99.

Moutinho, S., Martínez-Llorens, S., Tomás-Vidal, A., Jover-Cerdá, M., Oliva-Teles, A. and Peres, H., 2017. Meat and bone meal as partial replacement for fish meal in diets for gilthead seabream (Sparus aurata) juveniles: Growth, feed efficiency, amino acid utilization, and economic efficiency. *Aquaculture*, *468*, pp.271-277.

Nakada, M., 2008. Capture-based aquaculture of yellowtail. Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper, 508, pp.199-215.

Olsen, R.L. and Hasan, M.R., 2012. A limited supply of fishmeal: Impact on future increases in global aquaculture production. *Trends in Food Science & Technology*, *27*(2), pp.120-128.

Orellana, J., Waller, U. and Wecker, B., 2014. Culture of yellowtail kingfish (Seriola lalandi) in a marine recirculating aquaculture system (RAS) with artificial seawater. *Aquacultural engineering*, *58*, pp.20-28.

Pervin, M.A., Jahan, H., Akter, R., Omri, A. and Hossain, Z., 2020. Appraisal of different levels of soybean meal in diets on growth, digestive enzyme activity, antioxidation, and gut histology of tilapia (Oreochromis niloticus). *Fish physiology and biochemistry*, *46*, pp.1397-1407.

Ramezani, H., 2009. Effects of different protein and energy levels on growth performance of caspian brown trout, salmo trutta caspius (Kessler, 1877). *Journal of Fisheries and Aquatic Science*, *4*(4), pp.203-209.

Ramírez, C. and Romero, J., 2017. The microbiome of Seriola lalandi of wild and aquaculture origin reveals differences in composition and potential function. *Frontiers in Microbiology*, *8*, p.1844.

Randall, D.J. and Wright, P.A., 1987. Ammonia distribution and excretion in fish. *Fish physiology and biochemistry*, *3*, pp.107-120.

Ranjhan, R. and Athithan, S., 2015. Replacement of fish meal with canola/rapeseeds meal in aquaculture diets. *Int. J. Multidisciplinary Research and Dev*, *2*(1), pp.180-185.

Rašković, B., Stanković, M., Marković, Z. and Poleksić, V., 2011. Histological methods in the assessment of different feed effects on liver and intestine of fish. *Journal of Agricultural Sciences (Belgrade)*, *56*(1), pp.87-100.

Rema, P., Conceicao, L.E.C., Evers, F., Castro-Cunha, M., Dinis, M.T. and Dias, J., 2008. Optimal dietary protein levels in juvenile Senegalese sole (Solea senegalensis). *Aquaculture Nutrition*, *14*(3), pp.263-269.

Rossetti, N., 2012. The effect of dietary fish oil replacement with soybean oil on growth and health of dusky kob, *Argyrosomus japonicus (Pisces: Sciaenidae)*. Master's thesis). Rhodes University, Grahamstown, South Africa.

Rossi Jr, W. and Davis, D.A., 2014. Meat and Bone Meal as an Alternative for Fish Meal in Soybean Meal-Based Diets for Florida Pompano, Trachinotus carolinus L. *Journal of the World Aquaculture Society*, *45*(6), pp.613-624.

Sajjadi, M. and Carter, C.G., 2004. Dietary phytase supplementation and the utilisation of phosphorus by Atlantic salmon (Salmo salar L.) fed a canola-meal-based diet. *Aquaculture*, *240*(1-4), pp.417-431.

Salze, G.P., Stuart, K.R., Jirsa, D.O., Davis, D.A. and Drawbridge, M.A., 2018. Quantitative dietary taurine requirement for California yellowtail, Seriola lalandi. *Journal of the World Aquaculture Society*, *49*(1), pp.113-126.

Sankian, Z., Khosravi, S., Kim, Y.O. and Lee, S.M., 2017. Effect of dietary protein and lipid level on growth, feed utilization, and muscle composition in golden mandarin fish Siniperca scherzeri. *Fisheries and Aquatic Sciences*, *20*, pp.1-6.

Segner, H. and Witt, U., 1990. Weaning experiments with turbot (Scophthalmus maximus): electron microscopic study of liver. *Marine Biology*, *105*, pp.353-361.

Seo, B.S., Park, S.J., Hwang, S.Y., Lee, Y.I., Lee, S.H., Hur, S.W., Lee, K.J., Nam, T.J., Song, J.W., Kim, J.S. and Jang, W.J., 2022. Effects of Decreasing Fishmeal as Main Source of Protein on Growth, Digestive Physiology, and Gut Microbiota of Olive Flounder (Paralichthys olivaceus). *Animals*, *12*(16), p.2043.

Seo, B.S., Park, S.J., Hwang, S.Y., Lee, Y.I., Lee, S.H., Hur, S.W., Lee, K.J., Nam, T.J., Song, J.W., Kim, J.S. and Jang, W.J., 2022. Effects of Decreasing Fishmeal as Main Source of Protein on Growth, Digestive Physiology, and Gut Microbiota of Olive Flounder (Paralichthys olivaceus). *Animals*, *12*(16), p.2043.

Sitek, A.J., 2020. *Evaluating recirculating aquaculture system nutrient production* (Doctoral dissertation, University of New Hampshire).

Sofia, F., 2018. The State of World Fisheries and Aquaculture 2018-Meeting the sustainable development goals. *Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome*.

Steffens, W., 1989. *Principles of fish nutrition*. Ellis Horwood Limited.

Stephens, F.J. and Savage, A., 2010. Two mortality events in sea-caged yellowtail kingfish Seriola lalandi Valenciennes, 1833 (Nannopercidae) from Western Australia. Australian veterinary journal, 88(10), pp.414-416.

Sullivan, K.B., 2008. *Replacement of fish meal by alternative protein sources in diets for juvenile black sea bass* (Doctoral dissertation, University of North Carolina Wilmington).

Suloma, A., Mabroke, R.S. and El-Haroun, E.R., 2013. Meat and bone meal as a potential source of phosphorus in plant-protein-based diets for Nile tilapia (Oreochromis niloticus). *Aquaculture International*, *21*, pp.375-385.

Tacon, A.G., 1992. *Nutritional fish pathology: morphological signs of nutrient deficiency and toxicity in farmed fish* (Vol. 85, No. 22). Food & Agriculture Org.

Tacon, A.G. and Metian, M., 2013. Fish matters: importance of aquatic foods in human nutrition and global food supply. *Reviews in fisheries Science*, *21*(1), pp.22-38.

Thomas, P. and Robertson, L., 1991. Plasma cortisol and glucose stress responses of red drum (Sciaenops ocellatus) to handling and shallow water stressors and anesthesia with MS-222, quinaldine sulfate and metomidate. *Aquaculture*, *96*(1), pp.69-86.

Trushenski, J., Schwarz, M., Lewis, H., Laporte, J., Delbos, B., Takeuchi, R. and Sampaio, L.A., 2011. Effect of replacing dietary fish oil with soybean oil on production performance and fillet lipid and fatty acid composition of juvenile cobia Rachycentron canadum. *Aquaculture Nutrition*, *17*(2), pp.e437-e447.

Tun, M.M. and Swe, T., 2019. Experiment on growth response and Feed Conversion Ratio (FCR) of fishes by feeding BR Sludge from distillery spent wash at distillery factory in Yangon, Myanmar. *JAquacMarBiol* .2019;8(6):207-210. DOI: 10.15406/jamb.2019.08.00263

Webster, C.D., Tidwell, J.H., Goodgame, L.S., Yancey, D.H. and Mackey, L., 1992. Use of soybean meal and distillers grains with solubles as partial or total replacement of fish meal in diets for channel catfish, Ictalurus punctatus. *Aquaculture*, *106*(3-4), pp.301-309.

Williams, K.C., 2007. Optimum dietary protein and lipid specifications for juvenile malabar grouper (Epinephelus malabaricus). *Aquaculture*, *267*(1-4), pp.129-138.

Wilson, R.P., 2003. Amino acids and proteins. In *Fish nutrition* (pp. 143-179). Academic press.

Woolley, L., Jones, C.L.W. and Britz, P.J., 2009. The development of a practical diet for juvenile dusky *kob, Argyrosomus japonicus*, for the South African mariculture industry (Doctoral dissertation, Rhodes University).

Xu, Y. and Hanna, M.A., 2011. Nutritional and anti-nutritional compositions of defatted Nebraska hybrid hazelnut meal. *International journal of food science & technology*, *46*(10), pp.2022-2029.

Yang, M., Guo, X., Chen, T., Li, P., Xiao, T., Dai, Z. and Hu, Y., 2022. Effect of dietary replacement of fish meal by poultry by-product meal on the growth performance, immunity, and intestinal health of juvenile red swamp crayfish, procambarus clarkia. *Fish & Shellfish Immunology*, *131*, pp.381-390.

Ye, W., Han, D., Zhu, X., Yang, Y., Jin, J. and Xie, S., 2017. Comparative study on dietary protein requirements for juvenile and pre-adult gibel carp (Carassius auratus gibelio var. CAS III). *Aquaculture Nutrition*, *23*(4), pp.755-765.

Yigit, M., Erdem, M., Koshio, S., Ergün, S., Türker, A. and Karaali, B., 2006. Substituting fish meal with poultry by-product meal in diets for black Sea turbot Psetta maeotica. *Aquaculture Nutrition*, *12*(5), pp.340-347.

Zhang, S.Y., Li, G., Wu, H.B., Liu, X.G., Yao, Y.H., Tao, L. and Liu, H., 2011. An integrated recirculating aquaculture system (RAS) for land-based fish farming: The effects on water quality and fish production. *Aquacultural Engineering*, *45*(3), pp.93-102.

Zhou, Q.C., Mai, K.S., Tan, B.P. and Liu, Y.J., 2005. Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (Rachycentron canadum). *Aquaculture Nutrition*, *11*(3), pp.175-182.